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To the Graduate Council:

I am submitting herewith a dissertation written by Samuel Winfred Coleman entitled "N-Utilization by Cattle Fed Rations Consisting Mainly of Biuret and Low-Quality Hay and Factors Affecting N-Utilization by Cattle Fed Urea-Corn Silage Rations." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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N-UTILIZATION BY CATTLE FED RATIONS CONSISTING MAINLY OF BIURET
AND LOW QUALITY HAY AND FACTORS AFFECTING N-UTILIZATION
BY CATTLE FED UREA-CORN SILAGE RATIONS

A Dissertation
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
Samuel Winfred Coleman

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ABSTRACT

Two successive 7-day total-collection digestion and nitrogen (N) metabolism trials were conducted with beef steers to compare biuret with urea and to determine if other ration constituents influence utilization of N furnished by biuret. Twelve medium grade Hereford steers averaging 221 kg in body weight were allotted to one of the following treatments: 1) urea control; 2) biuret control; 3) biuret-molasses; and 4) biuret-corn. All animals received a basal ration of poor-quality fescue hay and were allowed 42 days to adapt to the NPN source before the start of the first trial.

A comparison of the data from the two trials indicated nutrient digestibility (dry matter, crude protein and gross energy) was not affected by NPN source. However, the addition of readily-fermentable energy (molasses, cornmeal) increased digestibility of all nutrients. Dry matter and gross energy digestibility of rations containing biuret and additional energy were significantly ($P < .10$) higher than of the urea and biuret control rations. Nitrogen digestibility was non-significantly higher in rations containing additional energy than in both control rations.

The percent absorbed N retained by animals consuming all types of biuret rations was lower than that by animals consuming the urea ration. The difference was from 6 to 8 percentage units, but was not significant ($P < .10$). Net protein utilization and percent intake N retained, which take into account digestibility, followed essentially the same pattern as percent absorbed N retained. They did, however,

reflect the advantage of additional energy on digestibility.

Another experiment was conducted to determine the pattern of peripheral blood ammonia and urea as a function of time after feeding. Six animals, used previously in metabolism trials, and six unadapted to NPN, were allotted to one of the following treatments: 1) urea-adapted; 2) biuret-adapted; 3) urea-unadapted; or 4) biuret-unadapted. Rations consisted of long timothy hay, blackstrap molasses and either urea or biuret. NPN comprised approximately 1.5 percent of the ration. Jugular blood samples were taken at intervals of 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours after feeding. An incomplete Latin Square design was used for sampling. Adapted animals consuming urea had the highest blood ammonia levels. Blood from animals of other treatments all contained approximately the same level of ammonia within all treatments, peak ammonia-N levels appeared at one hour after feeding. Plasma urea levels were fairly constant for all animals, averaging approximately 12-14 mg per 100 ml plasma. There was, however, a gradual increase in plasma urea from 2 to 6 hours.

Data from six total-collection digestion and N-metabolism trials with steers were compiled to examine some factors affecting ration digestibility and N-utilization of urea-corn silage based rations. F-max tests indicated homogeneity of variances among the trials. Multiple regression equations were developed to determine the effect of various ration constituents and intake parameters on nutrient digestibility and efficiency of N-metabolism. Calorie to protein ratio, grams N intake, percent digestible NFE and ration dry matter content together accounted

for 51, 46 and 74 percent of the total variability in the apparent digestibility of dry matter, crude protein and crude fiber, respectively. Increasing calorie to protein ratio resulted in a decrease of apparent digestibilities of dry matter and nitrogen, and an increase in digestibility of crude fiber. Nitrogen intake was associated negatively with digestibility. Increasing ration dry matter content increased dry matter and crude fiber digestibility and decreased that of crude protein.

When percent absorbed N retained, percent intake N retained and net protein utilization were the dependent variables, their regression on the four independent variables: 1) percentage of ration N supplied by urea; 2) energy supplied by concentrates; 3) N intake; and 4) fiber intake accounted for 34, 18 and 20 percent respectively, of the total variability in the three dependent variables. Percentage of N supplied by urea and N intake had no significant effect on any measure of N-metabolism. When energy supplied by concentrates increased from 3.8 to 11.3 mcal/day, retention of absorbed N, retention of intake N and net protein utilization increased 15.7 (significant, $P < .001$), 5.7 and 7.5 (significant, $P < .05$) percentage units, respectively. Increased fiber intake was associated with significantly ($P < .05$) decreased retention of intake N and net protein utilization. There was little effect of fiber intake on retention of absorbed N.

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CHAPTER I

INTRODUCTION

As the world population continues to increase, the competition between man and his domestic animals for the available food supply continues to increase. Monogastric animals like swine and poultry are in direct competition with man because they require similar food. However, ruminants like cattle and sheep can also live on feeds that cannot be utilized by man and monogastric animals. Forages are examples of feeds that can be utilized well only by ruminants. Also, in recent years the replacement of valuable plant protein meals by non-protein nitrogen (NPN) compounds in ruminant rations has contributed widely to the advancement of feeding a crowded world.

The use of NPN compounds, especially urea, as nitrogen (N) supplements in rations for ruminant animals has become a commonplace practice. Since the practice became prevalent following World War II, the use of urea as a feed item in the United States has increased to over 500,000 tons annually, replacing approximately 3,000,000 tons of plant protein meal. However, urea usually supplies only a small portion of total N intake. When higher levels of urea are added, the possibility of toxicity of the liberated ammonia exists. Further, utilization of the nitrogen is usually decreased and palatability of the ration is decreased.

Various NPN compounds other than urea have been investigated in an attempt to overcome some of the problems encountered with urea.

Among those tried was biuret--a condensation product of urea. Many of its properties, including low solubility in water and slow hydrolysis by ruminal biuretase, suggests that it might be useful as a nitrogen source for ruminants, especially in high-forage rations. One of the purposes of this study was to compare biuret and urea in maintenance-type rations containing low quality hay and to determine if the addition of corn or molasses affected N-utilization of these rations.

The present, wide-spread use of urea under many situations and previous research has suggested many factors which may affect optimum utilization of N from urea rations. Another purpose of this study was to determine the magnitude by which ration composition and intake factors are related to nutrient absorption and N utilization of steers fed rations composed mainly of urea and corn silage.

CHAPTER II

LITERATURE REVIEW

I. GENERAL ASPECTS OF NON-PROTEIN NITROGEN FEEDING

History of NPN Feeding

In 1891, Nathan Zuntz, a German nutritionist, suggested that rumen flora made possible the utilization of cellulose and nitrogen by ruminant animals (Stangel, 1967). For several years, this phenomenon was explored and the theories concerning the ability of rumen microbes to utilize non-protein nitrogen (NPN) were contested, primarily by Germans. Armsby (1911), working for the USDA, published a comprehensive review of work on the utilization of NPN by ruminants and deduced the role of microorganisms in converting NPN to protein, their subsequent digestion by the host animal and conversion to protein for milk and growth. He also pointed out some of the limitations in feeding NPN to ruminants.

Most of the research efforts concerning the use of NPN for ruminants were carried on by European workers, primarily Germans. The development of the Haber-Bosch synthetic ammonia process during World War I stimulated the successful development of a process to synthesize urea from ammonia and carbon dioxide on a commercial basis (Stangel, 1967). By 1930, the results of the European work, that ruminants were different in their protein requirements from other animals, began to spread to other parts of the world.

The first work in the United States was conducted at Wisconsin in the 1930's (Hart et al., 1939) with dairy animals and was followed by work at Illinois (Harris and Mitchell, 1941). Commercial production of urea in the United States, begun in 1935, made urea available at prices which allowed its use by feed manufacturers. Following this development, research programs in the United States concerning the use of urea and other NPN sources became prevalent. Positive results on the utilization of NPN in America were obtained shortly after World War II. The Wisconsin and Illinois work was followed by work at Massachusetts (Archibald, 1941) and at Cornell (Loosli, McCay and Maynard, 1942), and showed that significant amounts of urea nitrogen could be substituted for protein nitrogen in ruminant feeds with resulting growth and/or milk production (Garrigus, 1960).

Compounds Other Than Urea

Though urea has been the primary NPN compound used to substitute for preformed protein, other compounds have been tried and others will undoubtedly be tried in the future. Belasco (1954), using the artificial rumen, tried several urea derivatives such as methylene diurea, but found that these compounds failed to support cellulose digestion and produced poor bacterial growth. He also tried amides, which gave better results but were still inferior to urea. Other experiments, using ammonium salts, yielded some success with most of the salts promoting bacterial growth and subsequent cellular digestion. The ruminal ammonia level when these salts were fed was comparable to that when

urea was fed. Nitrogen from selected organic ammonium salts was better utilized than that from urea. Broster et al. (1960) and Tillman and Swift (1953) demonstrated in vivo, and Belasco (1954) and Hendrickx (1962) demonstrated in vitro that nitrogen from ammoniated products was utilized less than that from urea. They concluded that a high pH which resulted from using these compounds was responsible for the poor utilization. However, Hendrickx (1962) noted that ammonium sulphate increased in vitro protein synthesis by 80 percent over urea, possibly by some mechanism involving oxidation-reduction reactions in the rumen. Diammonium phosphate was shown by Oltjen et al. (1963) to be less toxic than urea but the nitrogen was not utilized as well. Another organic NPN compound, biuret, which is a condensation product of urea, has been studied as a possible source of nitrogen for ruminants (Garrigus, 1960; McLaren, 1964; Conrad and Hibbs, 1968; Chalupa, 1968; Oltjen, 1969; and Bartley, 1971).

Nitrogen Digestibility

The inclusion of urea in diets has usually resulted in higher apparent digestibility of nitrogen when compared to diets containing preformed protein (Barth, McConnel and Wang, 1968; Lowery and McCormick, 1969; and Oltjen et al., 1971). This is best explained by the rapid absorption of liberated ammonia through the rumen wall. The increase was proportional to the level of urea in the diet as demonstrated by Coleman, Shumway and Barth (1972) when urea furnished 24, 36 or 48 percent of the dietary nitrogen. However, when urea furnished either

0, 41 or 92 percent of the dietary nitrogen, Chalupa, Evans and Stillions (1964) noted that digestibility of nitrogen decreased with increasing urea.

Intake Problems

In a review, Chalupa (1970a) has suggested that substitution of urea for protein may result in feed intake depression. This is apparently due to the undesirable taste of urea when fed at certain concentrations as noted by Huber and Cook (1969). The concentrations at which sheep consistently refused solutions of urea have been observed by Goatcher and Church (1970) to be 2.5 to 5.0 percent. Van Horn, Foreman and Rodriguez (1966) observed that 1.9 percent urea in a concentrate mixture containing molasses was enough to depress intake by dairy cattle. The nature of the diet has profound influence on the effects of urea on voluntary intake. Feeding urea in corn silage (Van Horn et al., 1966), dehydrated alfalfa meal (Conrad, Hibbs and Neuhardt, 1969) and mixes of several ingredients as well as certain processing methods has been successful in at least partially overcoming consumption problems associated with urea feeding.

II. METABOLISM OF NON-PROTEIN NITROGEN COMPOUNDS

Ruminal Hydrolysis and Utilization

When urea is added experimentally to rumen contents, either in vivo or in vitro, the concentration of urea in the medium falls rapidly until urea is no longer detectable. The rate of urea hydrolysis was

observed to be 100 mg urea converted to ammonia per hour per 100 g rumen contents (Pearson and Smith, 1943). This represents the first stage in the process of utilization of urea-nitrogen by the animal and may be attributed to a potent urease enzyme produced in the rumen by several species and/or strains of bacteria (Pearson and Smith, 1943). The primary breakdown product from this hydrolysis is ammonia as shown by the addition of ^{15}N -ammonia to the rumen ammonia pool from ^{15}N -urea (Jones, 1967). It is reasonable to assume that most other NPN compounds are also reduced to ammonia since ammonia is the primary nitrogenous nutrient for rumen bacteria (Chalupa, 1970b).

Chalupa (1968) suggested that generally amination reactions are the primary mechanisms for ammonia assimilation by rumen bacteria since many keto acids are present in the rumen. One of the major problems, however, is the fact that normally ammonia release occurs very much faster than uptake and assimilation into microbial protein and results in an eventual loss of nitrogen available for microbial protein synthesis as shown by Bloomfield, Garner and Muhrer (1960). Thus, it would seem probable that a decrease in urea hydrolysis would increase assimilation of ammonia. Several methods have been used in an attempt to decrease the rate of hydrolysis. Among these are the administration of antibiotics (Prescott, 1953), and barbituric acid, copper and nitrate (Clifford, Bourdette and Tillman, 1968). Sidhu et al. (1969) injected jackbeam urease subcutaneously in an attempt to produce circulating urease antibodies. Urea hydrolysis was decreased and in most cases live-weight gains were increased.

Ammonia Absorption and Assimilation

Ammonia (common, toxic end-product of nitrogen metabolism) is absorbed primarily from the rumen but also from the omasum, lower part of the small intestine and cecum (Chalupa, 1969). The absorption rate is affected by both pH and concentration gradient of ammonia. Bloomfield et al. (1963) noted that since ammonia is a weak base, the increased absorption of ammonia at a higher pH is probably the result of an increase in the amount of NH_3 , which may penetrate the rumen mucosa more readily than NH_4^+ . The alkaline buffering capacity of rumen fluid is not as great as its acid buffering capacity. Thus, ruminal conditions are not only conducive to rapid ammonia production from urea, but also to increased absorption of ammonia (Chalupa, 1968).

Animals rid themselves of ammonia by synthesizing urea in the liver or by amination to glutamine, glutamic acid and other such compounds in the rumen mucosa, liver and other tissues (Austin, 1967). The synthesized urea is partially recycled through the saliva and rumen and the remainder is excreted. The inability of the liver to utilize all absorbed ammonia in these pathways is responsible for the appearance of ammonia in the peripheral blood which may result in toxicity (Chalupa, 1968). Toxic symptoms usually appear when peripheral blood ammonia levels reach a critical level of approximately 1 to 4 mg per 100 ml. These levels usually appear from one-half to one hour after urea administration. These symptoms include respiratory difficulties, excess salivation, muscular tremors, incoordination, bloat and death

within 1.5 to 2.5 hours after the initial appearance of symptoms (Clark et al., 1951; Repp et al., 1955; Oltjen et al., 1963; Austin, 1967; Chalupa, 1968). The primary cause of toxicity is excessive dietary urea which results in the rapid release and absorption of ammonia. However, it is difficult to determine the exact amount which causes toxicity, since other factors are also involved. Urea toxicity is more likely to occur if urea is administered suddenly such as by drench (Davis and Roberts, 1959, and Helmer and Bartley, 1971), or if the diet is deficient in digestible carbohydrate (Clark, Oyaert and Quinn, 1951), which is necessary for optimum utilization of liberated ammonia.

Nitrogen Retention in the Animal

Probably the factor which most inhibits a wider use of NPN is a decrease of nitrogen retention compared to nitrogen retention when proteins are fed. In a review, Oltjen (1969) noted that the growth rates and feed efficiency of animals receiving NPN as a major portion of their dietary nitrogen has been about 65 percent as good as those on protein containing diets. However, he points out that most of the results reported were from purified diets and that poor performance may well have resulted from nutritional inadequacy of the purified diet or the reduced intake on such diets.

Metabolism studies indicate that feeding high levels of urea rather than isolated soy protein in purified diets reduces retention of absorbed nitrogen by 30 to 40 percent (Oltjen and Putnam, 1966; Clifford and Tillman, 1968). In general, this decrease is influenced by

by the amount of total nitrogen that is replaced by urea. Chalupa, Evans and Stillions (1964) fed urea in semi-purified diets to furnish 0, 46 or 92 percent of the dietary nitrogen. There was a linear decline in percentage of absorbed nitrogen retained from 56.5 to 36.8 as urea nitrogen increased from 0 to 92 percent of the total. When corn silage formed a basal ration and was supplemented with urea to furnish 24, 36 or 48 percent of the ration nitrogen, Coleman, Shumway and Barth (1972) noted that the decrease in retention of absorbed nitrogen was curvilinear rather than linear. The values were 55.2, 51.8 and 44.6 percent of absorbed nitrogen retained when urea furnished 24, 36 and 48 percent of the dietary nitrogen respectively. Using multiple regression analysis, Smith et al. (1960) found that for each percentage unit increase in ration urea nitrogen, retention of absorbed nitrogen decreased 1.1 percentage units. Average retention was 45.6 percent and percent ration nitrogen supplied by urea ranged from 54.1 to 67.2 percent. Using similar techniques, Barth (1962) obtained a regression coefficient of 0.054 percentage units increase for each percentage unit increase in ration nitrogen supplied by urea. However, the percent of total nitrogen supplied by urea was higher (77.9 versus 62.8) and different variables were included in the models.

✓ Effect of Carbohydrate Source. Addition of starch to the diet may improve utilization as suggested both by in vitro (Belasco, 1956) and in vivo (Mills et al., 1944) studies of microbial protein synthesis.) Smith et al. (1957) showed that replacement of various amounts of wheat

straw with equal quantities of a mixture of dextrose and starch improved nitrogen utilization of urea rations by lambs. Other reports have also shown favorable response to addition of readily fermentable carbohydrates to urea rations (Fontenot et al., 1955 and Barth, 1962). This response is probably due to increased microbial protein synthesis as a result of increased amounts of intermediates of carbohydrate metabolism such as keto acids at the time of ammonia release (McLaren, 1964). These compounds may be used as carbon skeletons for amino acid synthesis.

Quality of Microbial Protein. Though it is well known that the nitrogen requirements of rumen microorganisms can be met by ammonia, some may require certain amino acids and growth stimulation of certain species has been noted with the addition of peptides to the medium (McLaren, et al., 1962). McLaren, Anderson and Barth (1965) noted that since ruminal bacterial and protozoal protein contain higher percentages of cystine and methionine, the quantity of sulfur-containing amino acids, especially methionine, required for protein synthesis may exceed the rate of production from urea nitrogen and biologically active precursors. Loosli and Harris (1945), Lofgreen, Loosli and Maynard (1947) and Gallup, Pope and Whitehair (1952) demonstrated an improvement in retention of absorbed nitrogen by lambs fed diets containing 30 to 40 percent of the nitrogen as urea and supplemented with methionine. McLaren, Anderson and Barth (1965) similarly showed that addition of either methionine or tryptophan

increased retention from 12 to 18 percent above that for basal-urea rations.

Since the use of automatic amino acid analyzers has become prevalent, the amino acid patterns of the rumen fluid and plasma have been studied. Slyter et al. (1971) noted that in most cases when urea was substituted for isolated soy protein in purified diets for cattle, significant decreases in blood plasma levels of leucine, isoleucine, valine and phenylalanine and increases in serine and glycine were observed. Oltjen and Putnam (1966) noted that these amino acid deficiencies were associated with reduced growth rates of the animals. In view of this, Oltjen et al. (1971) fed rations containing urea as the sole source of nitrogen and supplemented them with the sodium salts of the volatile fatty acid (VFA) precursors of amino acids 2-methyl butyrate, isovalerate, isobutyrate and phenylacetate. They generally noted slight increases in amounts of valine, leucine and isoleucine in the blood plasma. However, these amino acids, phenylalanine and total essential amino acids were still significantly lower in plasma when the urea diet was fed as compared to the soy-protein diet. There was an increase in N-retention when the isoacids were fed with either supplement, though the greatest response was with soy-protein diets.

Abomasal infusion of various amino acids was evaluated by nitrogen retention and indicated that limiting amino acids were methionine, lysine and threonine, in that order, on protein-free diets containing urea (Nimrick et al., 1970a). The maximum response was obtained when methionine, lysine -HCl and threonine made up 0.10, 0.16 and 0.10 percent

of the diet, respectively, in addition to 0.40 percent glutamic acid (Nimrick et al., 1970b).

Adaptation

✓ It is generally recognized that utilization of urea nitrogen improves the longer urea is fed. This phenomenon was observed by Reid (1953) and Repp et al. (1955), but the magnitude was not fully realized. Smith et al. (1960) demonstrated with multiple regression analysis that nitrogen utilization was affected by the length of time urea rations had been fed. They reported that retention of absorbed nitrogen increased 0.2 percentage units per day during a 50-day period when urea composed from 54 to 67 percent of the dietary nitrogen. When urea furnished essentially all the ration nitrogen, Barth (1962) observed that initial retention was lower and the response curve was steeper (0.4 percent per day) than in either of the above results.

The nature and site of adaptation response has not been well defined despite numerous attempts. Virtanen (1966) suggested that the low amount of rumen ammonia indicated adaptation by the rumen flora but Barth et al. (1961) were unable to detect increases in in vitro protein synthesis by rumen microorganisms obtained weekly from sheep fed all-urea supplement rations. Diethylstilbestrol has been demonstrated to shorten the period required to attain maximum utilization (McLaren et al., 1959; McLaren et al., 1960; Karr et al., 1965a). In a review, Chalupa (1968) has suggested that since diethylstilbestrol probably does not influence rumen microbial activity or digestibility its effect

must be on tissue metabolism. McLaren et al. (1961) demonstrated that rumen mucosal homogenates were able to synthesize L-glutamate and proposed that this might be the site of the adaptation response. However, McLaren, Cooper and Anderson (1962) discounted this when prolonged feeding of urea failed to influence the glutamic dehydrogenase activity of the rumen mucosa.

III. METABOLISM OF BIURET NITROGEN

General Aspects of Biuret Feeding

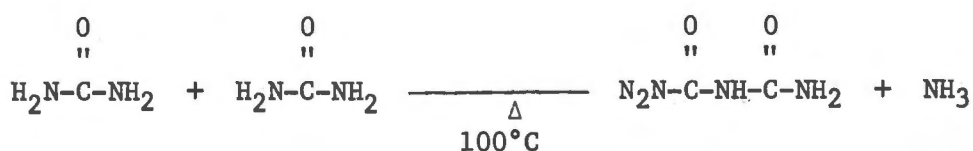
The previously-mentioned NPN compound, biuret, has received attention in attempts to overcome some of the problems of feeding urea. Several properties indicate that it may be beneficial in this respect.

Biuret is much less soluble than urea. This property should reduce the ammonia release rate, making biuret nitrogen available to rumen microorganisms at a time when more of the products of carbohydrate metabolism are available for amino acid synthesis. This property should also reduce the incidence of ammonia toxicity. Meiske et al. (1955), Repp et al. (1955) and Clark et al. (1963) drenched sheep with biuret ranging from 31.0 to 312.5 grams per 100 pounds of bodyweight and observed no symptoms of toxicity. Hatfield et al. (1959) fed, in one day, as much as 323 grams per 100 pounds body weight and noted distress as the only symptom of toxicity.

Biuret is also more stable than urea at high temperatures, such as those encountered in pelleting, cubing or blocking feeds. It is virtually tasteless, thus reducing the palatability or intake problem

experienced with urea. Ioset (1969) compared the intake of biuret to that of plant protein or urea. He reported that intake of concentrates containing biuret was slightly lower than that of protein but higher than that of urea.

Chemically biuret is a condensation product of urea and is formed when urea is heated to temperatures in excess of 100°C. The reaction involves two molecules of urea, forming biuret and releasing one molecule of ammonia as shown below.



In pure form, biuret contains 40.8 percent nitrogen. Feed grade biuret, which also contains urea, cyanuric acid and triuret in small quantities, contains at least 35 percent nitrogen and 219 percent crude protein equivalency.

Unlike urea, biuret is not hydrolyzed by ruminal urease. It is hydrolyzed to urea, carbon dioxide and ammonia by biuretase, an enzyme also produced by certain ruminal microbes. However, this enzyme is not constantly present and its production is only stimulated by the presence of biuret in the medium (Gilchrist, Potgieter and Voss, 1968).

✓ Similar to urea, biuret does not supply dietary energy to either microbes or the host animal. Hence, it is only used as a concentrated source of nitrogen.

Ruminal Hydrolysis and Microbial Utilization

The isolation of pure cultures of rumen microorganisms has been

a difficult task and, so far, very little is known about the total population. For this reason, identification of those organisms involved in metabolism of urea, biuret and other NPN compounds and the subsequent utilization of the ammonia produced has been a slow process. Slyter et al. (1968 and 1971) determined the effects of dietary nitrogen and carbohydrate source on the occurrence and ratios of rumen microbial species. They found that ruminal contents from steers fed biuret contained more facultative anaerobic bacteria than those from steers fed urea, uric acid or urea-phosphate. Protozoa numbers were less when animals consumed non-protein nitrogen containing semi-purified diets than when they consumed natural diets.

Biuret Hydrolysis. The elucidation of hydrolysis and utilization of biuret by rumen microbes can be attributed to workes in Illinois (Johnson and McClure, 1964), in South Africa (Gilchrist, Potgieter and Voss, 1968; and Schroder and Gilchrist, 1969) and with the Dow Chemical Company (Bauriedel et al., 1971). Ruminal contents were incubated with biuret and either the disappearance of substrate or appearance of product was used to determine if biuretolytic activity was present. Johnson and McClure (1964) failed to show an increase in the ammonia content of the medium or an appreciable net protein synthesis as a result of biuret breakdown. Gilchrist et al. (1968) on the other hand, demonstrated a significant disappearance of biuret from the medium which supported the growth of rumen microorganisms. Schroder and Gilchrist (1969) similarly demonstrated this but found

that straining ruminal ingesta through cheese cloth reduced the biuretolytic activity. This might explain the failure of Johnson and McClure (1964) to demonstrate significant protein synthesis from microorganisms in a biuret-containing substrate. Also, Bauriedel (1971) separated the fractions of the rumen ingesta into (1) upper-plant debris, (2) middle-bacteria and (3) lower-protozoa. Almost all of the biuretolytic activity was found in the portion of the rumen ingesta containing plant debris, indicating a close association between biuret-hydrolyzing organisms and particulate matter. Isolation of a crude cell-free extract also showed a concentration of biuretase activity in the plant debris portion of the rumen fluid. Measurement of urease activity indicated that it (urease) was more generalized in location, although results were inconclusive.

End Products. The end products of biuret hydrolysis were observed to be carbon dioxide and ammonia by Bauriedel (1971) and Bauriedel et al. (1971) using isotope techniques. Also, a small amount of labeled carbon from biuret was recovered as methane, especially if the media contained natural feedstuffs or starch and cellobiose (Bauriedel, 1971). Further analysis of the microorganisms and certain amino acids revealed labeling of these fractions. The amino acids containing the highest ^{14}C activity were glutamate and aspartate. Bauriedel et al. (1971) demonstrated the appearance of ^{15}N in the ammonia fraction and in both microbial protein and free amino acids.

Effect of Carbohydrate Source. Since the presence of readily-available energy has a significant effect on the retention of urea nitrogen (Fontenot et al., 1955 and Barth, 1962), it is of interest if the type of energy fed to animals has an effect on biuret hydrolysis and utilization. Gilchrist et al. (1968) noted that in vitro breakdown of biuret increased when maize (corn) meal was added to the diet of the animal. However, Schroder and Gilchrist (1969) failed to obtain this response by adding starch to the medium. They even observed a decrease in biuretolytic activity, but attributed it to changes in pH. Better retention of the ammonia produced was noted when donor animals received a good quality hay. This was probably due to the greater availability of carbohydrate in the better quality forage. Bauriedel (1971) and Bauriedel et al. (1971) found a decrease of labeled elements in the measured CO_2 and NH_3 , and an increase of these elements in the microbial mass as a result of adding starch and cellobiose to the medium. This suggests that addition of carbohydrate may not increase biuret hydrolysis but does increase bacterial proliferation and incorporation of end products into microbial protein. This also demonstrates that the appearance of ammonia in a system is a poor measure of hydrolysis.

Adaptation. As with urea, there appears to be some type of adaptation to most NPN compounds. Apparently, the mechanisms of adaptation to the various compounds are different, yet very little is known about these mechanisms. Biuret also requires an adaptation period before optimum utilization can be expected. This response was observed

by early workers, e.g. Welch et al (1957), Ewan, Hatfield and Garrigus (1958), Hatfield et al. (1959), McLaren et al. (1959), Campbell et al. (1963) Johnson and McClure (1964), McKenzie and Altona (1964a) and Clark, Barrett and Kellerman (1965). Gilchrist et al (1968), demonstrated that in vitro biuretolytic activity was evoked only when biuret was included in the diets of the animals from which the rumen fluid was taken. In examining this phenomenon further, Schroder and Gilchrist (1969) noted a lag from the time biuret was introduced into the diet until the biuretolytic activity appeared. These results and those reported by Bauriedel (1971) suggest that in the case of biuret, the adaptation response may be due to microbial activity rather than any response of the animal. This is probably due either to an increase in numbers of organisms which produce biuretase or by induction of the enzyme by the substrate and is different from the adaptation to urea in which case urease is constantly present.

Adaptation by microorganisms may also be influenced by the type of ration the animal receives. Schroder and Gilchrist (1969) noted that the number of days required for development of maximum biuret hydrolysis was a function of crude protein content of the basal ration. The higher the protein content of the basal rations (teff hay), the longer the period before maximum biuretase activity occurred. Another interesting point was the immediate decrease in biuretolytic activity in rumen contents when biuret was removed from the diet of donor animals.

To summarize, it appears that biuret is broken down by microbial enzymes to carbon dioxide and ammonia. Biuretase is different from urease because it either must be induced or the biuretase producing bacteria must be propagated, and because of its close proximity to the particulate matter in the rumen. However, urease may be required for complete hydrolysis of biuret. The energy source apparently does not affect actual hydrolysis but does influence utilization of liberated ammonia.

Nitrogen Retention in the Animal

The ammonia released by bacterial hydrolysis of biuret may follow one of several pathways including: (1) incorporation into bacterial protein while still in the rumen; (2) amination to amino acids by the rumen mucosa; (3) absorption through the rumen wall into the blood stream, and either (a) recycled through the saliva; or (b) excreted in the urine. The rate of ammonia liberation would affect which of the above mechanisms predominate in the removal of ammonia from the rumen, with slower release favoring the first pathway. It is in this way, that biuret, at least in theory, would be favorable as an NPN source.

Adaptation. Early reports were conflicting with respect to the feeding value of biuret as a nitrogen source for ruminants when compared to urea. Meiske et al. (1955) noted that lambs consuming a basal ration composed of approximately 50 percent corn, 30 percent wheat straw, 7 percent molasses and 0.85 percent biuret were equal to those consuming

similar rations with 0.85 percent urea when body weight gains and feed efficiency were the criteria. Gaither et al (1955) reported that nitrogen from biuret was retained by lambs as well as that from urea, and Campbell, Warner and Loosli (1960) noted no difference in urea or biuret for milk production by dairy cattle. However, Hatfield et al. (1959) noted that steers consuming prairie hay supplemented with soybean meal, urea or biuret retained less nitrogen when the supplement was biuret.

More recent experiments by Karr et al. (1965a); Karr et al. (1965b); Slyter et al. (1971) and Chicco et al. (1971) demonstrated greater nitrogen utilization of biuret than or urea. However, Oltjen et al. (1968 and 1969) found lower utilization of biuret using semi-purified diets and Clark, Barrett and Kellerman (1963) and Schaadt, Johnson and McClure (1966), found essentially no differences. These results support early work and show clearly that discrepancies exist in data comparing these two compounds. Several trials were undertaken to study the reasons for these discrepancies (Ewan, Hatfield and Garrigus, 1958; Anderson et al., 1959; Hatfield et al., 1959; and McLaren et al., 1960), with the results indicating that an adaptation period is also required for optimum utilization of biuret nitrogen. Oltjen (1969) mentioned that this adaptation may be similar to that associated with feeding urea. It is likely, however, that a large portion of the adaptation response when feeding biuret is the result

of stimulation of the production of biuretase, whereas urease is constantly present and urea hydrolysis is usually very rapid (Schroder and Gilchrist, 1969). Similarities in response for the two compounds could be conditioning of microbes to utilize ruminal ammonia better and conditioning of the animal to use more absorbed ammonia in the rumen mucosa and liver for amino acid synthesis to glutamine, glutamic acid and other compounds.

Ewan et al. (1955) demonstrated that the addition of inoculum from animals consuming biuret to the rumen of test lambs increased the percentage nitrogen intake retained (biological value), indicating a response of "adapted" microorganisms. McLaren et al. (1959) and Karr et al. (1965a) found that diethylstilbestrol (DES) increased the utilization of biuret nitrogen. This response is probably exhibited by the animal since DES does not affect rumen microbes. This response is probably similar to that obtained with urea diets. Schaadt, Johnson and McClure (1966) found a decrease in urinary biuret excretion with time, but failed to obtain response due to adaptation in either nitrogen balance or retention of absorbed nitrogen.

Effects of Energy Source. Since biuret has a slower ammonia release than urea, the release pattern should be more compatible to that of carbon precursors from forages which can be used for amino acid synthesis. Oltjen et al. (1968), using purified diets, presented data obtained from rumen samples in support of this, but nitrogen retention of steers fed biuret was slightly lower than of those fed

urea.. Also fiber digestibility was depressed from 45 percent with urea to 26 percent with biuret. They suggest the release of ammonia may be too slow to support fiber digestion. In support of this, Slyter et al. (1971), also using purified diets high in fiber, demonstrated that addition of starch increased nitrogen retention almost 100 percent when biuret was the NPN source and approximately 20 percent when urea was the nitrogen source. Both supplements were approximately equal when no starch was added. Also, Clark, Barrett and Kellerman (1965) feeding teff hay, observed low retention values from biuret not supplemented with corn meal as compared to urea, biuret, triuret and cyanuric acid, all supplemented with corn meal.

Feeding Trials With Biuret

The favorable results obtained, at least in part, from balance and metabolism studies have led to feeding trials to determine whether biuret could be favorably utilized for growth and fattening of ruminant animals. Meiske et al. (1955) demonstrated that feeding biuret resulted in gains similar to those obtained from feeding urea or soybean meal in diets based primarily on corn and wheat straw. Hatfield et al. (1959) observed larger gains from biuret-supplemented rations than from urea-supplemented rations for growth and reproduction of sheep when the NPN furnished two-thirds of the total nitrogen. Similar results were obtained by Karr et al. (1965a), Chicco et al. (1971) and Clanton (1972) by feeding NPN at the rate of 60, 30 and 17 percent of the ration

nitrogen, respectively, and by Oltjen et al. (1969) when a twice daily feeding regime was used rather than ad libitum feedings. Biuret seems to have a distinct advantage when the basal ration contains a high degree of forage such as low quality range forage (Raleigh and Turner, 1968), timothy hay (Oltjen et al., 1969), green-chopped elephant grass (Chicco et al., 1971) or corn silage (Karr et al., 1965b). Of particular interest is the trial by Karr et al. (1965b) in which part of NPN was ensiled and part was added at feeding time. Average daily gains of lambs consuming rations containing approximately 20 percent of the dietary nitrogen as urea or biuret added at feeding were 168 and 173 grams respectively, whereas they were 173 and 191 for urea and biuret, respectively when the NPN sources were ensiled. However, Meiske, Goodrich and Owens (1969) reported values which were just opposite of this with steers. Biuret ensiled at the rate of 0.55 percent yielded the lowest gains and urea administered in the supplement yielded the highest, although differences were non-significant. They also compared silage rations containing biuret and urea for growing and fattening cattle. The best gains on the growing ration were made by animals consuming urea whereas gains on the finishing diets were better from animals consuming biuret. A more complete adaptation may have taken place during the fattening phase which resulted in improved performance at that time, or the gains may have been compensatory in nature. Overall gains were approximately equal for both diets.

In many cases, cattle and sheep are allowed to "rough it" on over-ripe dry ranges or over-winter on mature forage which is low in

crude protein content. It becomes necessary to provide a low-cost protein supplement in a form which requires minimum labor, since labor costs for feeding often exceed the cost of supplement. Cow-calf operations common in the Southeast lend themselves to this. The ideal type would be a mineral lick or block such as that used in South Africa (MacKenzie and Altona, 1964a, and MacKenzie and Altona, 1964b) and on a Utah winter range (Fonnesbeck, Harris and Kearl, 1971), at least when gains and feed efficiency are the criteria. The use of urea in such supplements increases the chance for toxicity and low utilization may result due to rapid hydrolysis of urea and lack of readily fermentable carbohydrate. It is in this situation that biuret should, in relation to its properties, exhibit the best results as a non-protein nitrogen source and find an important use in the cattle industry.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. DIGESTION AND NITROGEN METABOLISM TRIAL WITH BIURET

Experimental Design

Two successive digestion and N-metabolism trials were conducted, with 12 beef steers in each trial. The steers were allotted to one of four experimental rations consisting of a low quality hay and either urea control, biuret control, biuret and molasses, or biuret and block simulation mixture according to body weight and condition at the beginning of the experiment and remained within that treatment group for the duration of the experiment.

Animals

Twelve Hereford beef steers of Medium condition grade and averaging 221 kg in body weight were selected from the University of Tennessee-Atomic Energy Commission herd at Oak Ridge and used in this experiment. Prior to the experiment, the animals were fed rations consisting primarily of corn silage.

Rations

All animals were offered a ration of low-quality fescue and broomsedge hay, which had an average protein content of 6.7 percent at the rate of about 4.5 kg per head daily. The hay was supplemented with

one of four concentrate supplements containing either urea or biuret as a source of nitrogen (Table I). The diet contained a crude protein equivalency of approximately 9 percent, which is slightly less than the recommendations of NRC for growing beef cattle gaining at the rate of 0.25 kg per day. The protein content was intentionally low so that maximum available protein would be used. The animals received their respective ration from the onset of the experiment.

Conduct of Trials

The animals were allotted to treatment groups and placed in individual metabolism crates (Hobbs, Hansard and Barrick, 1950). A blood sample was taken from each animal for blood ammonia and urea analysis on day 0, which represents the day before NPN was fed. Forty-two days (adaptation period) were allowed for the animals to adapt to their respective NPN source, the rations in general and the environment. Additional blood samples were obtained during this period at 1, 7, 21 and 35 days after feeding NPN. The animals were fed equal parts of the total ration intake twice daily. Water and trace-mineralized salt was provided ad libitum. The animals were removed from the crates and weighed on day 38.

After the adaptation period, two successive 7-day collection periods were conducted. Feces were collected daily and a 5 percent sample from each animal was composited and refrigerated at 5°C in jars containing thymol to retard mold formation. Total daily urinary excretion of each steer was collected in jars containing 25 ml 6 N HCl

TABLE I
RATION CONSTITUENTS OFFERED, KG/DAY^a

Item	Treatment			
	Urea control	Biuret control	Biuret molasses	Biuret corn
Trial I				
Hay	6.4	6.4	6.0	6.0
Molasses, blackstrap	0.10	0.10	0.45	---
Corn, ground shelled	---	---	---	0.35
Urea (45% N)	0.068	---	---	---
Biuret (39%)	---	0.082	0.086	0.078
Trial II				
Hay	6.1	6.1	5.7	5.7
Molasses, blackstrap	0.10	0.10	0.45	---
Corn, ground shelled	---	---	---	0.35
Urea (45% N)	0.068	---	---	---
Biuret (39%)	---	0.082	0.086	0.078

^aT-M salt and steamed bonemeal were fed free choice.

and a pinch of thymol to prevent spoilage. The urine for each animal was brought to a constant volume (4,000 ml), which was established as the maximum daily excretion of any one steer during the preliminary period, by the addition of tap water. A daily sample of 10 percent of the constant volume was taken, composited for each steer with 6N HCl and thymol to prevent spoilage, and refrigerated at 5°C until analyzed.,

Laboratory Analysis

Aliquot fecal samples from each animal were thoroughly mixed after each collection period and a 500 g wet sample was dried for 72 hours in a forced-air oven at 65°C. The samples were allowed to air-equilibrate for 48 hours, re-weighed, ground through a medium-mesh screen (2 mm) of a Wiley Mill and placed in air-tight jars until analyzed.

Samples of ration components, refusals and feces were analyzed in duplicate for nitrogen and moisture, and urine was analyzed in triplicate for nitrogen according to A.O.A.C. (1965) methods. Molasses were analyzed in duplicate for moisture by the toluene distillation method (A.O.A.C., 1965). Gross energy content of the ration, refusals and feces were obtained in duplicate according to Parr Instrument Company (1960) adiabatic calorimeter procedures.

Blood ammonia levels were determined by the micro-diffusion technique of Conway (1950) and urea by the method of Van Slyke and Cullen (1914). Creatinine and creatine levels of the urine were assayed using the method described by Oser (1965). Urinary urea was subjected

to urease, and the resulting ammonia was determined by its reaction with sodium phenate and hypochlorite⁼ to form a blue color as described by Carver (1971).

Computations and Statistical Analysis

Apparent digestion coefficients were calculated for dry matter, crude protein and gross energy. True digestibility of crude protein was calculated after fecal nitrogen excretion was adjusted for metabolic N (MFN) by using the formula: $MFN = 11.9 \times \text{kg fecal DM}$ (Swanson and Herman, 1943). Percent absorbed N retained was calculated using the Thomas-Mitchell method after adjusting urinary N for endogenous N (EUN). EUN was calculated using the equation $EUN = 0.45 \text{ wt}_{gk}^{.49}$ (Swanson, E. W., unpublished data). Net protein utilization (NPU), expressed as percent, is the product of true N digestibility and percent absorbed N retained/100. Net protein value (NPV) indicates the percent utilizable crude protein in the ration and is the product of NPU and the ration crude protein/100. An unadjusted estimate of overall utilization, percent intake N retained, is the quotient of N retained (g/day) and N intake (g/day) $\times 100$.

The data were analyzed statistically to determine the effects of treatment and period (trial) on retention and absorption of nitrogen and absorption of other nutrients. A split-plot design was used (Snedecor, 1956). Orthogonal contrasts (Table II) were used to separate treatment differences into single groups.

TABLE II
ORTHOGONAL CONTRASTS USED TO SEPARATE
TREATMENT DEGREES OF FREEDOM

Contrast	Treatment			
	Urea control	Biuret control	Biuret molasses	Biuret corn
I. Urea versus biuret	+1	-1	0	0
II. Molasses versus corn	0	0	+1	-1
III. Energy versus no energy	+1	+1	-1	-1

II. BLOOD PARAMETERS

Experimental Design

Twelve beef steers were used to determine the pattern of whole blood ammonia and plasma urea levels as a function of time after feeding either urea or biuret. Three steers each were assigned to the following treatments: (1) urea, adapted for 56 days; (2) biuret, adapted for 56 days; (3) urea unadapted; and (4) biuret, unadapted. The adapted animals had previously been used in the metabolism study.

The design used for collecting blood is shown in Table III. Subcells consist of animal number within each treatment.

Rations

All animals received long timothy hay ad libitum. The hay averaged 8.3 percent crude protein on a dry basis. The NPN source was fed twice daily in a slurry with blackstrap molasses. The composition of rations can be seen in Table IV.

Blood Sampling and Analysis

Two jugular blood samples were obtained from each steer daily, using heparinized disposable syringes and 18 gauge needles. The blood was taken immediately to the laboratory, a 0.6 ml aliquot removed for ammonia determination and the remainder centrifuged at 2000 g for 15 minutes. The plasma was removed by siphon and frozen at -20°C until analysed for urea. Ammonia was analyzed on the whole blood using the

TABLE III
DESIGN USED FOR COLLECTING JUGULAR BLOOD SAMPLES

Time after feeding	Day		
	1	2	3
15 minutes	1 ^a	2	3
30 minutes	2	3	1
1 hour	3	1	2
2 hours	1	2	3
4 hours	2	3	1
6 hours	3	1	2

^aAnimal number.

TABLE IV
RATIONS OF ANIMALS USED FOR BLOOD NITROGEN STUDY^a

Constituent	Treatment			
	Urea adapted	Biuret adapted	Urea unadapted	Biuret unadapted
Timothy hay, long, kg/day	5.7	5.8	5.7	5.3
Molasses, blackstrap, kg/day	0.42	0.42	0.42	0.42
Urea, kg/day	0.08	---	0.08	---
Biuret, kg/day	---	0.091	---	0.091
Nitrogen, %	1.86	1.81	1.86	1.86
NPN, %	1.29	1.44	1.29	1.56
NPN, % of total nitrogen	31.0	29.2	31.0	31.0

^aDry matter basis.

micro-diffusion technique of Conway (1951). Plasma urea was determined by the colorimetric procedure described by Carver (1971), which is based on the reaction of ammonia, liberated by urease, with sodium phenate and hypochlorite to produce a blue color.

III. FACTORS AFFECTING N-METABOLISM OF CORN

SILAGE AND UREA RATIONS

Conduct of Trials

Data from 55 steers in six different digestion and nitrogen-metabolism trials which were similar in manner of conduct and in ration components were compiled to study the effects of several variables on nutrient digestibility and on retention and utilization of nitrogen. A summary of the ration intake and ration component data is presented in Table V. Basal rations were composed of corn silage with various amounts of urea either ensiled or added at feeding time.

Animals were confined to individual metabolism crates (Hobbs, Hansard and Barrick, 1950) for 10-day preliminary and 7-day collection periods. They were fed twice daily and excreta was collected once daily. An attempt was made to keep silage fresh. If it was removed from the silo at any time other than immediately before feeding, it was stored at 5°C in steel drums. Silages and feces were sampled, aliquots composited and refrigerated until the end of a trial. They were then mixed thoroughly and dried in a forced-air oven at 65°C. Ration, refusal and fecal samples were ground through a medium mesh screen of a Wiley Mill and stored in air-tight glass jars for subsequent

TABLE V

SUMMARY OF MEAN OBSERVATIONS OF RATION CONSTITUENTS AND INTAKE PARAMETERS OF ANIMALS
USED IN TRIALS FROM WHICH MULTIPLE REGRESSION EQUATIONS WERE OBTAINED

Trial	Number of animals	Ration constituents ^a						Ration nitrogen %	N supplied by urea %	Dry matter intake kg/day
		Corn silage %	Corn cob meal %	Alfalfa hay %	Ground- shelled corn %	Urea %	CSM %			
H72T8-1	2	27.2	45.3	18.2	6.32	1.47	---	2.29	28.9	4.62
H72T8-2	3	33.2	55.4	---	7.73	1.80	---	2.09	38.7	3.78
H72K2-2	4	41.0	---	9.11	37.3	0.69	11.9	2.27	32.3	4.58
H72K2-3	4	40.6	---	9.18	37.6	0.70	12.0	2.38	30.6	4.55
H72K2-4	4	29.3	---	10.2	53.9	0.54	5.06	2.53	22.3	4.23
H72K2-6	4	31.0	---	10.2	53.5	0.54	5.02	2.83	21.1	4.26
H71K3-1	4	38.4	---	---	55.9	0.55	5.13	2.08	28.8	3.89
H71K3-2	4	36.4	---	---	60.2	1.12	2.10	1.63	47.4	4.17
H71K3-3	4	31.8	---	---	65.1	1.79	1.37	1.98	62.7	4.15
H71K3-4	4	42.1	---	---	50.1	0.72	7.09	2.01	27.6	4.37
H71K3-5	3	48.3	---	---	46.9	1.28	3.50	1.87	41.5	4.65
H71K3-6	4	46.5	---	---	52.0	1.99	---	1.90	61.8	4.48
H71K3-7	4	75.4	---	---	15.8	1.38	7.34	2.64	24.3	3.30
H71K3-8	4	74.9	---	---	19.4	2.06	3.76	2.63	34.8	3.32
H71K3-9	3	74.5	---	---	23.0	2.92	---	2.67	45.4	3.35
Mean ^b		44.9	51.4	10.6	40.9	1.25	5.90	2.27	37.1	3.92

^aDry matter basis.

^bMean of those included.

analysis. Feeds, feces, refusal and urine samples were analyzed for crude protein and feeds and feces were analyzed for proximate components (A.O.A.C., 1965). Gross energy was determined on feeds and feces using a Parr bomb adiabatic calorimeter (Parr Instrument Company, 1960).

Calculations

From the data collected, apparent digestion coefficients for all nutrients were calculated. Further, true digestibility of nitrogen was calculated by adjusting fecal nitrogen for metabolic fecal nitrogen (MFN) (Swanson and Herman, 1943). Efficiency of intermediary N metabolism was estimated by calculating percentage of absorbed N retained by the Thomas-Mitchell method. Endogenous urinary nitrogen (EUN) was calculated by the formula: $EUN = 0.45 \text{ wt}_{\text{kg}}^{.49}$ (Swanson, E. W., unpublished data). Net protein utilization was calculated by multiplying percent absorbed N retained by the true digestibility/100. An unadjusted estimate of overall utilization, percent intake N retained, was calculated by dividing grams N retained by the grams N intake x 100.

Statistical Analysis

Since the data were taken from six different trials under slightly different conditions, a test for homogeneity of variances was conducted using the F-max test. Simple correlation coefficients were calculated among digestion coefficients, measures of N-utilization, nutrient intake and several other variables. Multiple regression techniques according

to Draper and Smith (1966) were used to study the influence of intake, various ration constituents and general animal performance on nutrient digestibility and N-metabolism. Equations were fitted using an IBM 360/65 computer and a program prepared by Barr and Goodnight (1971). The general form of the regression equations was as follows:

$$\hat{Y}_1 = a + b_1X_1 + b_2X_2 + \dots + b_jX_j$$

The intercept (a) is calculated by the formula:

$$a = \bar{Y} + \sum_j b_j (0 - \bar{X}_j).$$

The b_j 's are the coefficients of partial regression of the dependent variable on the independent variables.

The X_j 's are the independent variables measuring nutrient intake, ration characteristics and/or animal performance.

The $\hat{Y}_1$'s are the estimated values of dependent variables for specified values of the X_j 's. These dependent variables include nutrient digestibility and various measures of N-metabolism.

The $\bar{Y}_1$'s are the means of dependent variables.

The $\bar{X}_j$'s are the means of the independent variables.

CHAPTER IV

RESULTS AND DISCUSSION

I. N-METABOLISM TRIALS WITH BIURET

In both collection trials, total dry matter intake was lower than NRC (1970) requirements for steers weighing 221 kg and gaining 0.25 kg per day (Table VI). The hay, selected for its low crude protein content, apparently was not sufficiently palatable to the animals, since there was considerable refusal. During the preliminary-adaptation period, the hay was chopped with a hammermill, hopefully to improve consumption. Three steers in each trial were discarded because of refusal of NPN compounds, poor separation of feces and urine and/or sickness.

The two different trials were conducted to determine if complete adaptation to the NPN compounds had occurred. Ration intake data and nutrient digestibility for each trial are presented in Table VII. Dry matter intake was slightly lower in trial II. This may have resulted from "metabolism cage fatigue" or from the increased environmental temperature which occurred during the second period. However, nutrient digestibility was essentially unchanged from trial I to trial II.

Nitrogen utilization data for each trial are presented in Table VII. Previous results have indicated that retention and utilization of N from NPN sources are generally lower before complete adaptation has occurred. In the present study, there was no increase of any parameter of nitrogen retention between trials I and II. Therefore, adaptation to both urea and biuret had apparently occurred before

TABLE VI
RATION INTAKE AND COMPOSITION

Item	Treatment			
	Urea control	Biuret control	Biuret molasses	Biuret cornmeal
Trial I				
Ration intake, kg/day ^a	3.95	3.90	4.08	3.95
Nitrogen, %	1.67	1.82	1.68	1.74
Hay, %	96.16	96.04	89.40	90.30
Blackstrap molasses, %	2.07	2.02	8.66	---
Ground shelled corn, %	---	---	---	7.90
Urea (45% N), %	1.77	---	---	---
Biuret (36.8% N), % ^b	---	1.94	1.94	1.80
Salt (trace mineralized)	+	+	+	+
Steamed bonemeal	+	+	+	+
Trial II				
Ration intake, kg/day ^a	3.42	3.74	3.85	3.90
Nitrogen, %	1.74	1.87	1.67	1.71
Hay (6.7 protein), %	95.73	95.88	88.62	90.01
Blackstrap molasses, %	2.30	2.11	9.30	---
Ground shelled corn, %	---	---	---	8.13
Urea (45% N), %	1.97	---	---	---
Biuret (36.8% N), % ^b	---	2.01	2.08	1.86
Salt (trace mineralized)	+	+	+	+
Steamed bonemeal	+	+	+	+

^aDry-matter basis.

^bFeed grade.

TABLE VII

COMPARISON OF RATION INTAKE, NUTRIENT DIGESTIBILITY
AND N-UTILIZATION BETWEEN TRIALS

Item	Trial	
	I	II
Number of animals	9	9
Number of days from first feeding of NPN ^a	45	52
Ration intake, kg/day ^b	3.97	3.75
Total N of ration, %	1.75	1.72
Apparent digestibility, %		
Dry matter	50.2	49.5
Nitrogen	55.1	54.0
Gross energy	47.6	48.3
Nitrogen, gm/day		
Intake	68.5	64.7
Fecal	30.8	29.8
Absorbed	37.7	34.9
Urinary	31.1	31.4
Retained	6.61	2.34
Metabolic fecal ^c	23.5	22.5
Endogenous urinary ^d	6.55	6.55
True N digestibility, % ^e	89.4	88.9
N retained, % absorbed ^f	59.7	54.8
N retained, % intake	9.65	3.62
Net protein utilization, % ^g	53.4	48.7

^aMeasure is from first day of feeding to the middle of the trial.

^bDry-matter basis.

^cMFN = $11.0 \times \text{kg DM feces}$ (Swanson and Herman, 1943).

^dEUN = $0.45 \text{ wt}_{\text{kg}}^{.49}$ (Swanson, E. W. 1971, unpublished data).

^eTrue N digestibility = $\frac{\text{intake N} - (\text{fecal N} - \text{MFN})}{\text{intake N}} \times 100$.

^fThomas-Mitchell biological value of protein.

^gNet protein utilization = $\frac{\text{true N digestibility} \times \text{absorbed N retained}}{100}$.

trial II. These data support the findings of McLaren et al. (1959), Johnson and McClure (1964) and Oltjen et al. (1969) who suggested that complete adaptation for biuret occurs between 28 and 40 days, and those of Oltjen et al. (1969) who noted that steers consuming high forage diets adapted to urea within seven days. Since no statistical differences due to trial were observed (Appendix Tables XVI and XVII) data were pooled to examine treatment effects.

Nutrient Digestibility

The nutrient digestibility data, pooled from both trials, are shown in Table VIII. Dry matter and gross energy digestibility were not affected significantly ($P < .10$) by NPN source (urea control versus biuret control). Anderson et al. (1959), Schaadt, Johnson and McClure (1966) and Oltjen et al. (1969) also observed that high forage rations containing either biuret or urea were similar in dry matter digestibility. However, addition of either molasses or ground shelled corn to the ration significantly ($P < .10$) increased dry matter digestibility and gross energy digestibility by approximately 5 to 8 percentage units. The higher digestibilities of dry matter and energy of these rations are reasonable since most of the energy in molasses and corn is more highly digestible than that of forages.

Apparent nitrogen digestibility was slightly but non-significantly ($P < .10$) higher in rations containing either additional molasses or corn. The differences were not as pronounced as those in dry matter or gross energy digestibility. Nitrogen digestibility of the urea control

TABLE VIII
EFFECT OF TREATMENT ON NUTRIENT DIGESTIBILITY AND
NITROGEN UTILIZATION OF A DIET CONTAINING
LOW QUALITY HAY^a

Item	Treatment			
	Urea control	Biuret control	Biuret molasses	Biuret corn
Number of animals	3	2	2	3
Weight gain, kg/day	0.19	0.24	0.37	0.13
Digestibility, %				
Dry matter	46.6 ^h	46.2 ^h	52.3 ⁱ	51.4 ⁱ
Nitrogen	52.6	52.3	55.5	55.9
Gross energy	44.9 ^h	43.6 ^h	55.6 ⁱ	49.8 ⁱ
N balance, gm/day				
Intake N	64.27	68.36	69.00	65.47
Fecal N	29.40	32.61	30.69	29.01
Absorbed N	34.87	35.75	38.30	36.46
Urinary N	27.26	32.80	32.88	33.67
Retained N	7.61	2.96	5.42	2.79
Metabolic fecal N ^b	22.75	23.68	23.39	22.39
Endogenous urinary N ^c	6.48	6.43	6.67	6.60
True N digestibility, % ^d	89.3	86.9	89.3	90.0
N retained, % absorbed ^e	63.7	55.6	57.0	57.5
N retained, % intake	9.97	4.34	7.37	4.65
Net protein utilization, % ^f	56.9	48.3	51.0	51.8
Net protein value, % ^g	5.62	4.59	4.86	4.71

^aMeans of both trials.

^bMFN = $11.9 \times \text{wt}_{\text{kg}} \text{ fecal dry matter}$.

^cEUN = $0.45 \text{ wt}_{\text{kg}}^{.49}$.

^dTrue N digestibility = $\frac{\text{intake} - (\text{fecal} - \text{MFN})}{\text{intake}}$

^eThomas-Mitchell biological value of proteins = $\frac{\text{intake N} - (\text{fecal N-MFN}) - (\text{urinary N-EUN})}{\text{intake} - (\text{fecal N-MFN})}$.

^fNPU = % absorbed N retained x true digestibility/100.

^gNPV = NPU x % crude protein of ration.

^{h,i}Values in the same line having different superscripts are significantly different ($P < .10$).

ration was essentially the same as that of the biuret control ration. Campbell, Warner and Loosli (1960) and Oltjen et al. (1969) also observed essentially no differences in the N digestibility of rations containing urea or biuret as N supplements. However, McLaren et al. (1959) and Schaadt, Johnson and McClure (1966) observed significantly higher N digestibility of semi-purified rations containing urea than of those containing biuret.

Nitrogen intake variability (Table VIII) reflects variability in dry matter intake. Though the rations were calculated to be isonitrogenous, differential intake of the hay resulted both in variable N concentration and N intake. The highest N intake was observed in the ration containing biuret and molasses. Animals consuming rations containing biuret and molasses also absorbed the greatest amount of N. This may be accounted for by the greater N intake since the fecal excretion of nitrogen was not as variable as the intake. The high fecal N excretion by animals consuming the biuret control diet may have resulted from passage of biuret into the lower gastrointestinal tract and excretion, rather than hydrolysis by rumen microbes.

Nitrogen Utilization

Animals consuming rations containing biuret excreted more urinary nitrogen than those consuming the ration containing urea, resulting in less nitrogen retained per day. However, differences were not statistically significant ($P < .10$) among rations. Nitrogen retention by animals consuming the biuret-molasses ration was numerically greater than that of animals consuming biuret control or biuret-corn rations.

Generally, the amounts of nitrogen retained were very variable within all treatments, and one animal, assigned to the biuret control ration had a negative balance (Appendix Table XVII). Several other animals eating various rations retained less than one gram of N per day.

When N retained is calculated as percent N absorbed, the values varied less. Animals consuming the urea control ration retained approximately 8 percentage units more (64 versus 56 percent) than animals consuming the biuret control ration. However, this difference was not significant ($P < .10$). These data agree with those of Oltjen et al. (1969) who supplemented timothy hay with biuret or urea and noted that animals consuming biuret retained less nitrogen than those consuming urea. However, Clark, Barrett and Kellerman (1965) and Schaadt, Johnson and McClure (1965) found essentially no difference in daily nitrogen retention by sheep fed biuret or urea, and Chicco et al. (1971) observed that animals eating green-chopped elephant grass (6.7% crude protein) retained more N from biuret than from urea.

The addition of readily-fermentable carbohydrates in the form of molasses or ground shelled corn to the biuret ration resulted in a slight increase in the percentage of absorbed N retained when compared to the biuret control ration. Karr et al. (1965a) hypothesized that increasing the propionate production in the rumen might favor N utilization of urea, and the same hypothesis may be valid for biuret. Further, Gilchrist et al. (1968) noted that in vitro biuret hydrolysis increased with the addition of cornmeal, and Schroder and Gilchrist (1969) attributed better N utilization by animals consuming good quality hay to higher levels of readily-available carbohydrates in the hay.

In general, it appears that the values for percent absorbed N retained were high for all treatments. However, the rations in this study were low in crude protein and it is well known that animals consuming rations slightly deficient in crude protein content retain a higher percentage of absorbed nitrogen than those fed rations adequate or excessive in nitrogen content. Smith et al. (1960) noted that with each percentage unit decrease in ration N, percentage of absorbed N increased 1.07 units.

Net protein utilization values, determined by multiplying the percent absorbed N retained by the true N digestibility, show a pattern similar to that of retention of absorbed N, since true N digestibility was similar for all treatments. The biuret control ration yielded the lowest percent net protein utilization and the urea control ration yielded the highest, with those containing biuret and readily available energy being intermediate. The numerical differences observed were not significant ($P < .10$).

Comparison of protein values of the rations indicates that the urea control ration contained the highest percentage of utilizable N but differences among treatments were not significant. Those rations containing biuret were similar, but the addition of energy increased net protein value slightly.

Urinary Nitrogen Fractionation

Urinary N was partitioned in an attempt to study the relationship of the fractions with N utilization. The results are presented in

Table IX, with data from the two trials having been pooled. There were no statistical differences ($P < .10$) among treatments in the amount of any urinary fractions. Urea comprised a major portion of the total urinary nitrogen, which is similar to the results reported by Chalupa, Evans and Stillions (1964). However, in the present study, undetermined N accounted for a greater percentage of the total N than in previous studies, and changes in the amount of total urinary N are closely paralleled by changes in undetermined N. When biuret was the NPN source, the undetermined N may have resulted from urinary biuret excretion. Gray and Clark (1964) observed that sheep excreted from 7 to 22 grams of biuret per day when dosed with 50 grams of biuret while consuming a teff hay ration. Oltjen et al. (1968) reported that cattle excreted approximately 10 grams of biuret N per day when biuret intake was 90 g/day but Hatfield et al. (1959) reported a biuret nitrogen excretion of approximately 5 g per day when steers were fed approximately the same amount of biuret. When 70-80 g of biuret were fed per day in the present study, values of undetermined N were within this range.

The excretion of creatinine and creatine N, expressed as percentage of total urinary N, was lower in the present study than in the study by Chalupa, Evans and Stillions (1964). The difference may be accounted for by the type of ration, e.g., natural in the present study and semi-purified in the former. It was assumed that differences in creatinine excretion were due to animal variation and sampling error since diet generally does not affect the excretion of creatinine (Oser,

TABLE IX

INFLUENCE OF TREATMENT UPON URINARY NITROGEN DISTRIBUTION^a

Component	Treatment			
	Urea control	Biuret control	Biuret- molasses	Biuret- corn
Total urinary N, gm/day	27.26	32.80	32.88	31.75
Nitrogen partition:				
Urea + NH ₃ N, gm/day	20.68	22.68	21.54	24.02
Creatinine N, gm/day	0.11	0.54	0.59	0.63
Creatine N, gm/day	0.78	0.61	0.69	0.91
Undetermined N, gm/day	5.69	8.97	10.06	6.19
% of total urinary N				
Urea + NH ₃ N	75.87	69.15	65.51	75.65
Creatinine N	0.40	1.65	1.79	1.98
Creatine N	2.86	1.86	2.10	2.87
Undetermined N	20.87	27.35	30.60	19.50

^aData pooled from two trials.

1965). Chalupa, Evans and Stillion (1964) noted that an increase in creatine N excretion was associated with weight loss of the animals on high urea diets and attributed the increased creatine to tissue catabolism due to inferior diets when large amounts of urea were fed. However, this relationship did not appear to exist in the present study.

The excretion of urea plus ammonia N from animals consuming all of the biuret rations was higher than that from animals consuming urea-control rations, even though it was less in biuret rations when expressed as a percentage of the total urinary N. Generally, urea plus ammonia N excretion paralleled that of total urinary N, but undetermined N was also increased when biuret was fed.

Conclusion

In conclusion, animals consuming maintenance rations of low-quality hay and urea utilized numerically but non-significantly ($P < .10$) greater percentage of N than animals consuming similar rations containing biuret. Further, there were no indications that urea fed with such rations and at these levels was toxic, even to unadapted animals. The addition of energy to those rations containing biuret slightly increased N utilization.

II. BLOOD NITROGEN PARAMETERS

During the preliminary-adaptation period of the metabolism trials reported earlier, jugular blood samples were taken to determine if

peripheral blood ammonia and urea levels showed evidence of adaptation to NPN sources when cattle were consuming poor quality forage as a maintenance type ration. The data from these samples are presented in Figures 1 through 4, with each figure representing one of the four treatments. At no time did the levels of ammonia of an animal reach levels which approach toxicity. Animals from treatments I and II (urea control and biuret control) were first fed the NPN in a salt-mineral mix but consumption was poor and on the tenth day a small quantity (50 g/feeding) of molasses were fed to stimulate intake. The next blood sample, taken on the twenty-first day reflected this by containing the peak concentration of ammonia. There were no differences in ammonia-N concentration among any treatments if the peak concentration for each is considered.

Urea-N levels were affected very little by treatment from 0-35 days of NPN feeding. The levels were similar to those reported by Oltjen et al. (1968) and Slyter et al. (1971) who fed steers high-fiber semi-purified diets for 40 days. There was a slight peak on day 1 (the day after feeding NPN) for all treatments, and after day 7 there was a gradual increase in concentration of urea N. The animals consuming urea had slightly higher blood urea concentration. After 35 days, blood taken from all animals averaged about 14 mg urea-N per 100 ml whole blood.

Though ammonia-N levels of animals from all treatments decreased from day 21 to day 35, levels on day 35 were very similar to those on day 0. Therefore, apparently the animals had adapted to either utilize

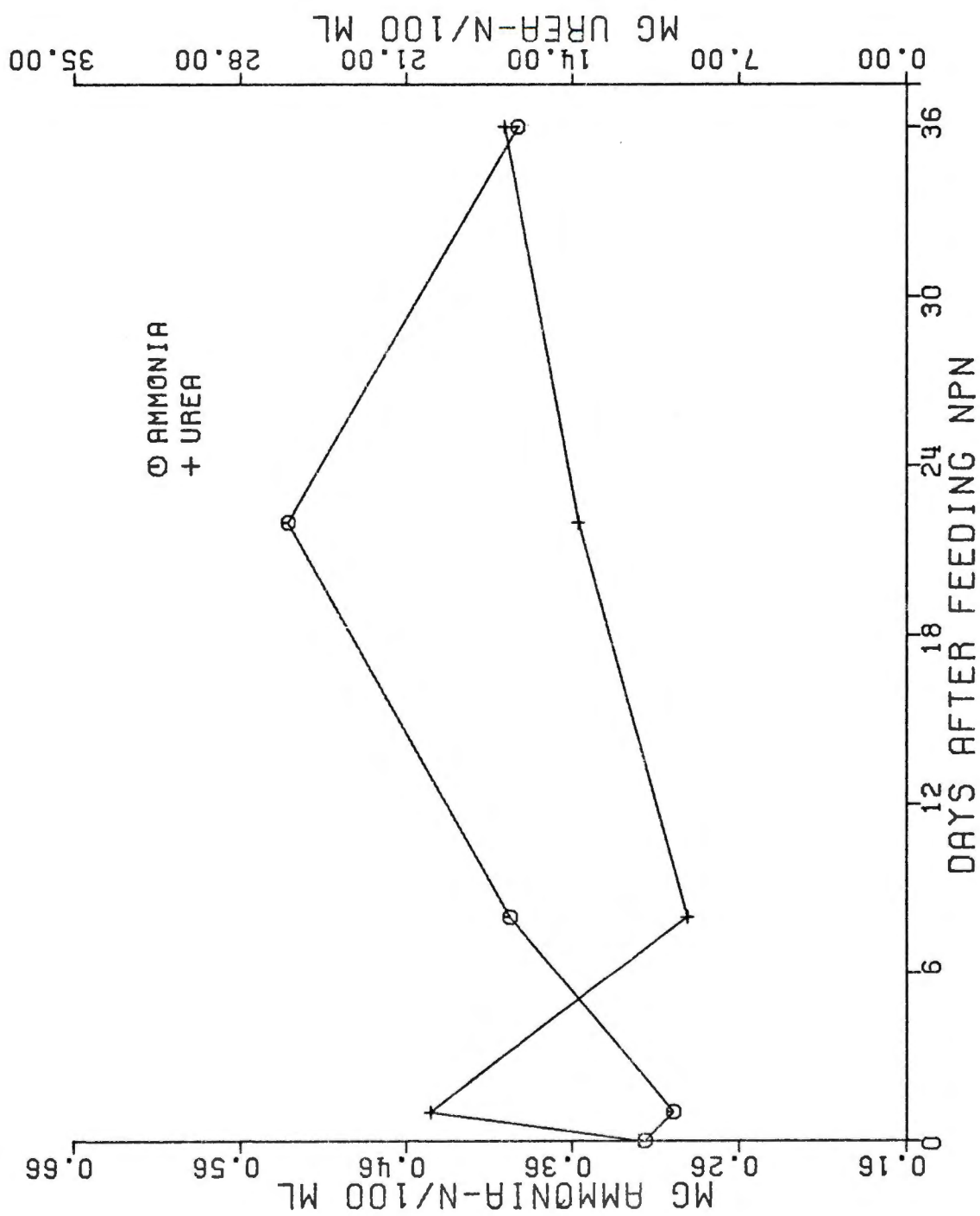


FIGURE 1. Whole blood ammonia and urea levels of animals fed urea control ration.

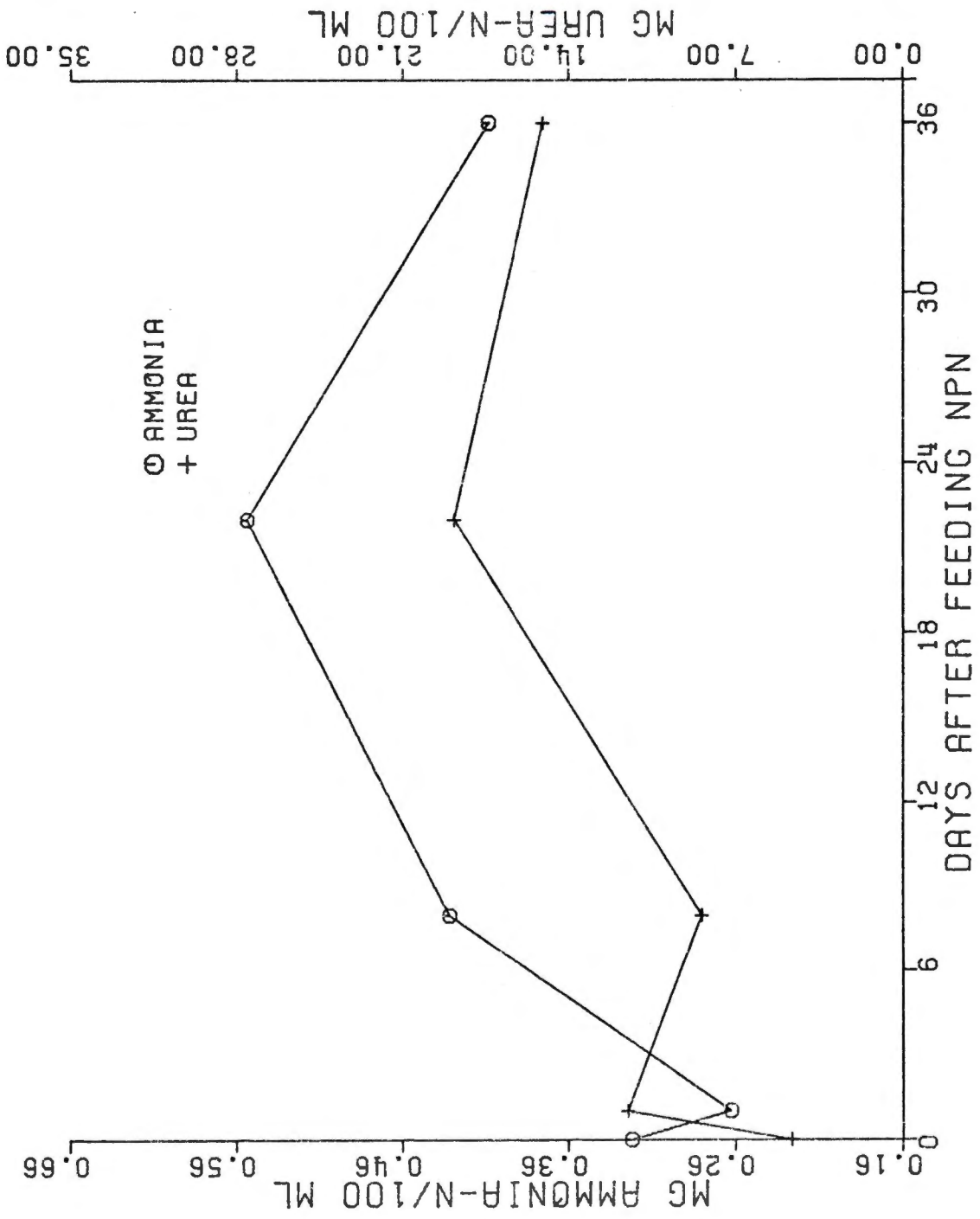


FIGURE 2. Whole blood ammonia and urea levels of animals fed biuret-control ration.

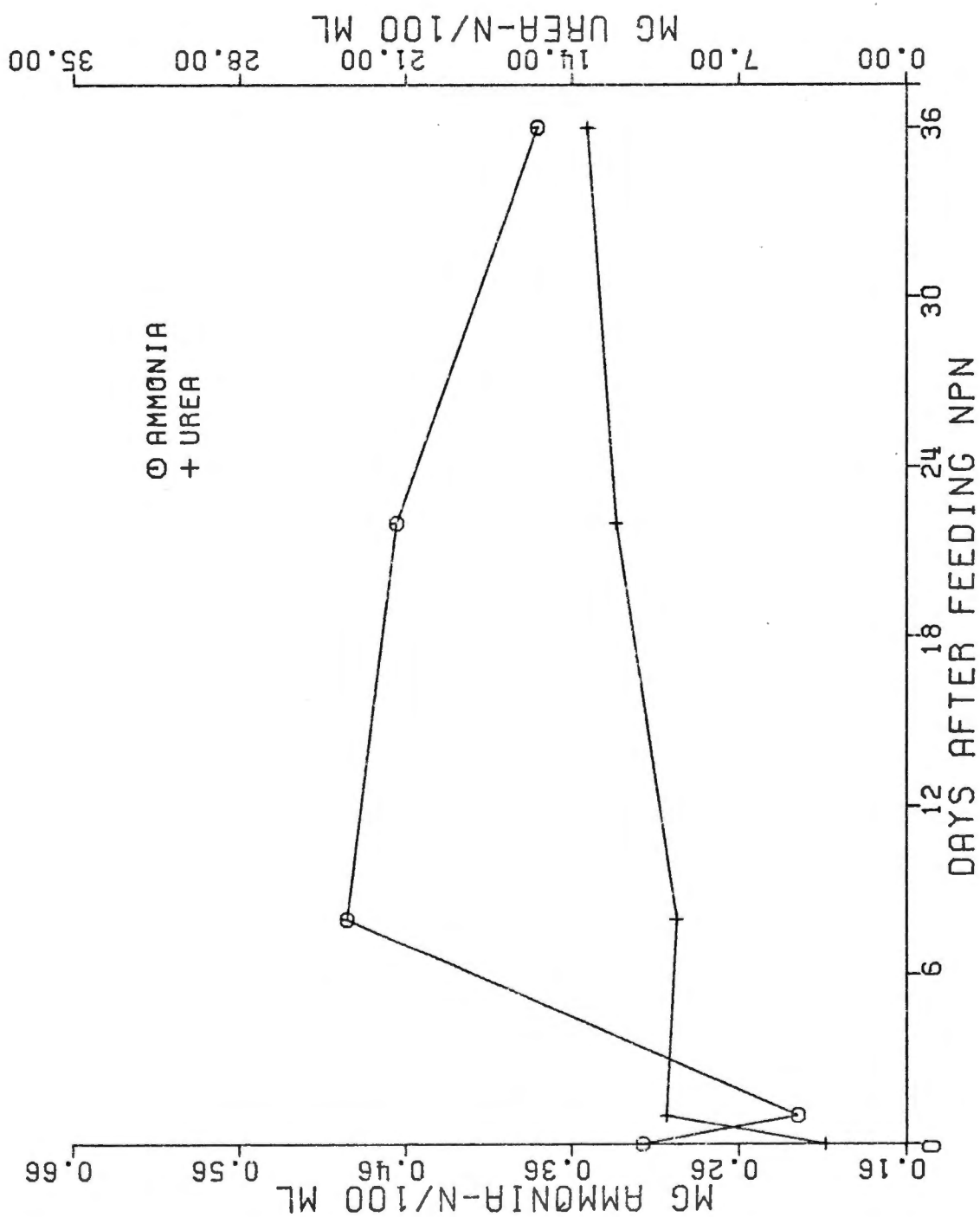


FIGURE 3. Whole blood ammonia and urea levels of animals fed biuret-molasses ration.

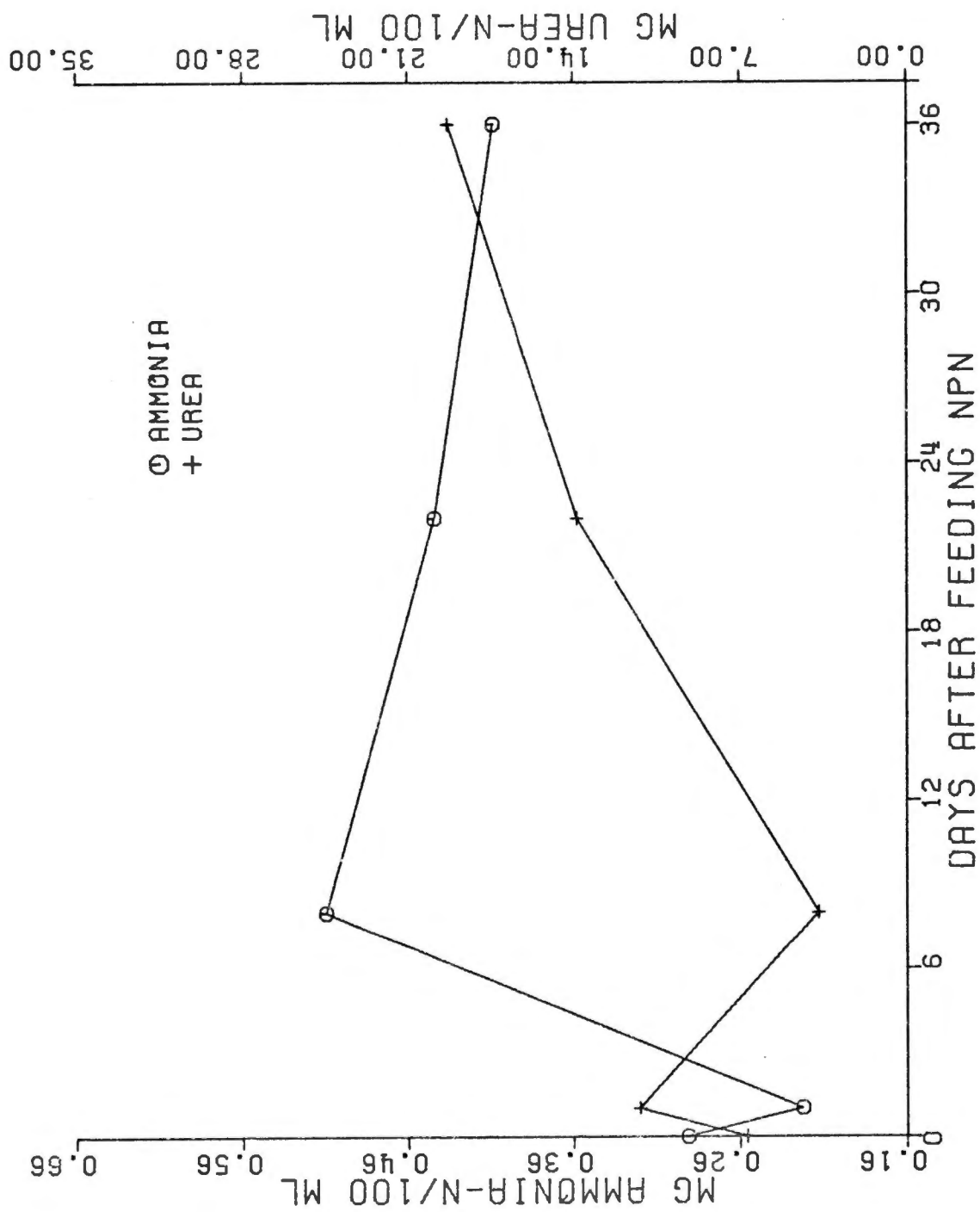


FIGURE 4. Whole blood ammonia and urea levels of animals fed biuret-corn ration.

the ammonia as it entered the blood or to utilize it via microflora before it was absorbed. The slight increases in urea N levels might indicate that conversion to urea might be at least one mode of adaptation. However, conversion of all measurable ammonia of the peripheral blood to urea would increase urea only slightly, since the ammonia-N concentration is a very small fraction of the urea-N concentration.

Since ammonia and urea-N levels differed little among treatments, it was felt that possibly two hours after feeding was not the time at which different levels of blood ammonia and urea could be detected. A search of the literature revealed that little information was available concerning the blood ammonia and urea pattern with time after feeding. Therefore, immediately following the N-metabolism trials, six of the animals were removed and replaced with six unadapted ones for a study of blood nitrogen parameters from 15 minutes to 6 hours after feeding. Means for the four treatments are plotted in Figures 5 through 8.

The results show that again, relatively low concentrations of blood ammonia-N were observed for all animals. These values (.25 to .60 mg/100 ml blood) are similar to those observed in sheep by Carver (1971) when urea composed of 1-2 percent of the ration. Rations in the present study contained approximately 1.5 percent NPN. It is not understood why animals adapted to urea for approximately 55 days had higher blood ammonia-N values than those which were unadapted. The animals of this treatment were used previously in the metabolism trial and the change from broom-sedge hay to timothy hay may have stimulated

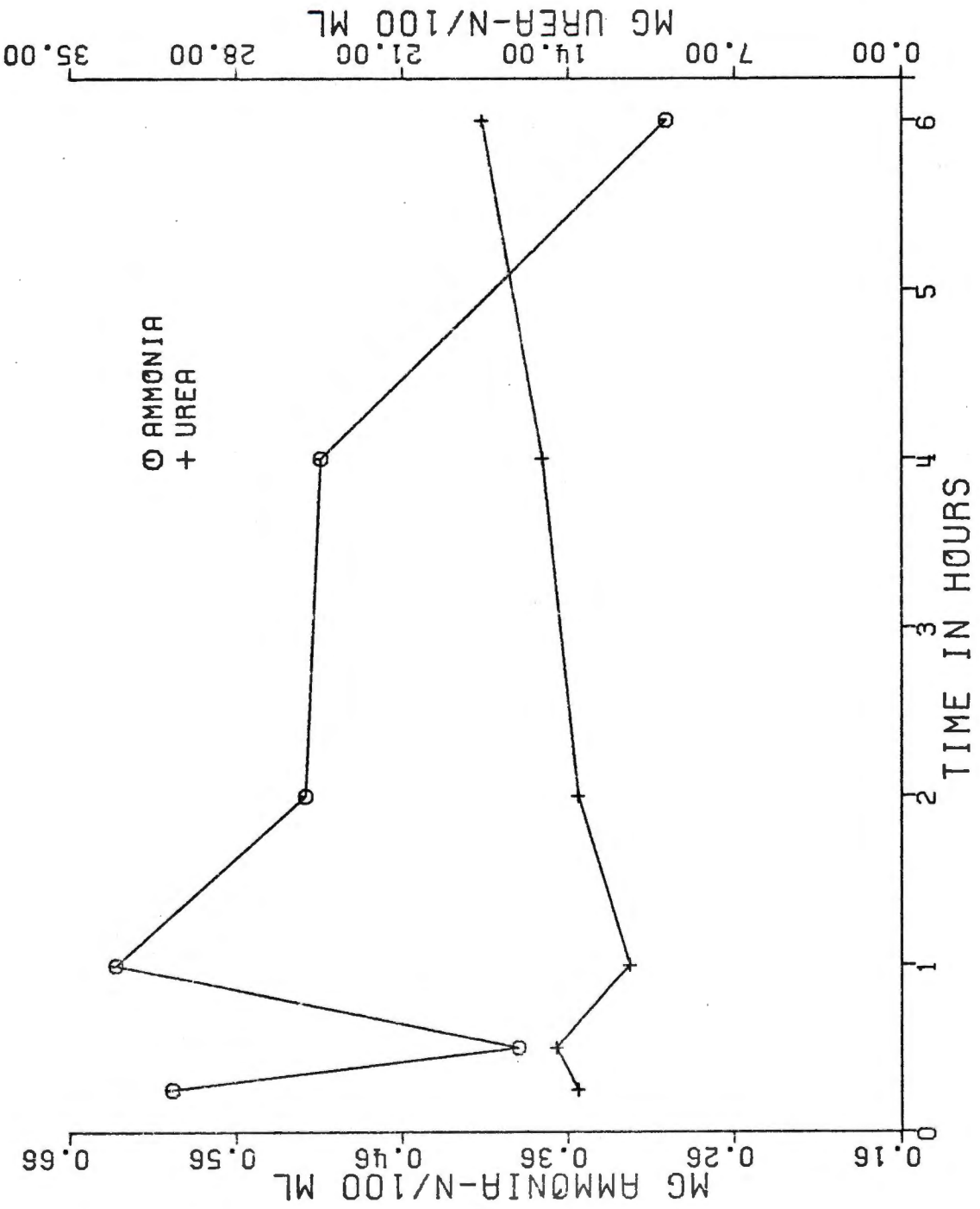


FIGURE 5. Whole blood ammonia and plasma urea of urea adapted animals fed urea.

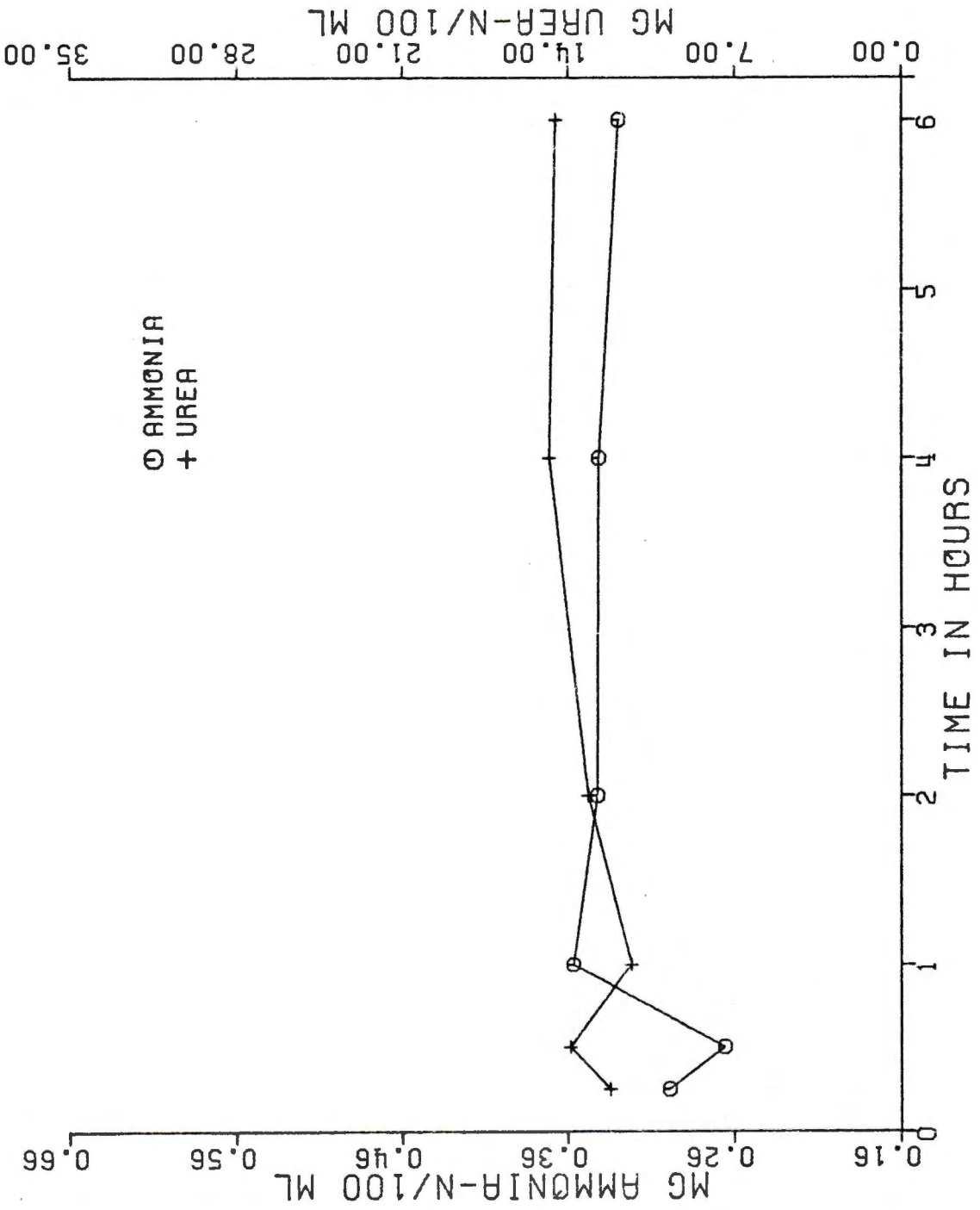


FIGURE 6. Whole blood ammonia and plasma urea of biuret adapted animals fed biuret.

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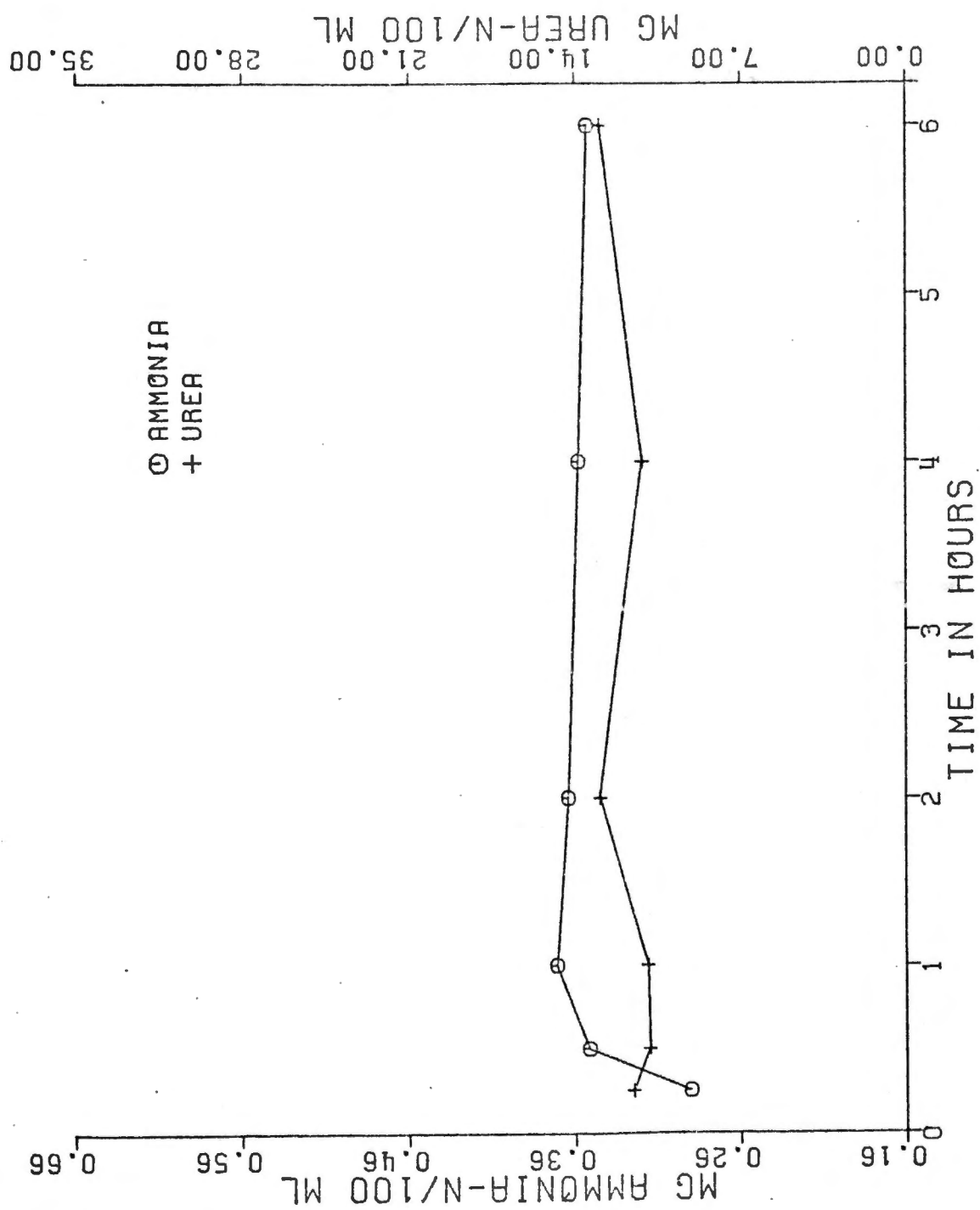


FIGURE 7. Whole blood ammonia and plasma urea of unadapted animals fed urea.

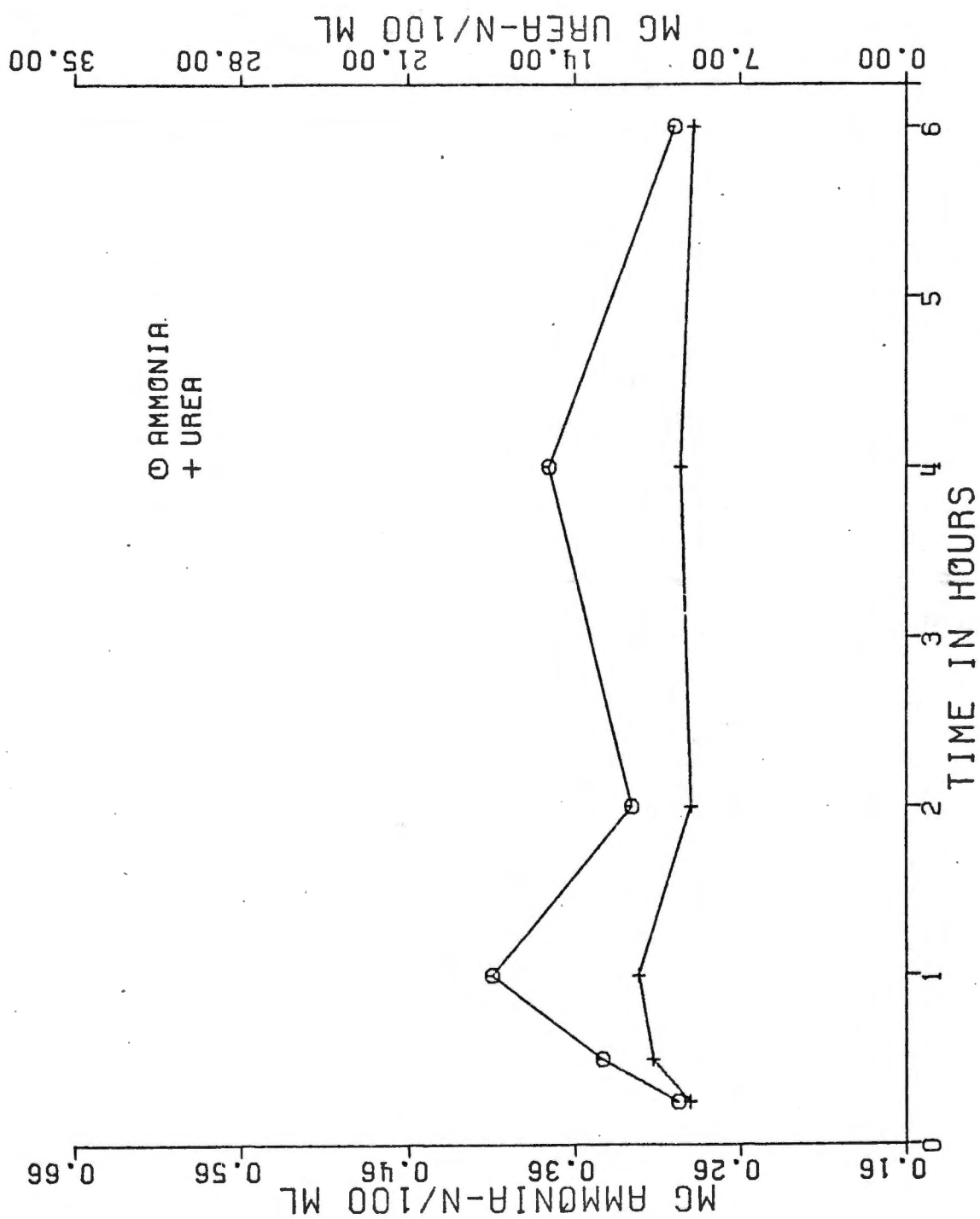


FIGURE 8. Whole blood ammonia and plasma urea of unadapted animals fed biuret.

further metabolic changes in animals which had been previously adapted, resulting in higher values.

In most cases, the peak ammonia level was obtained at one hour, which is earlier than the blood samples in the previous study were taken. This peak is similar to those observed in sheep by Carver (1971). Also, previously reported ruminal ammonia peaks, which usually occur from 15 minutes to 1 hour after feeding or dosing (Oltjen et al., 1968; Carver, 1971) most probably would result in peak blood levels at approximately one hour. Repp et al. (1955) drenched sheep with toxic levels of urea and noted that when blood ammonia-N was a function of time, concentration rose 28.3 micrograms per 100 ml. blood for each minute after dosing. Urea levels also increased but at much slower rates.

At one hour after feeding, adapted animals consuming urea had whole blood ammonia-N levels of approximately 0.6 mg/100 ml whereas those consuming biuret had blood ammonia levels of approximately 0.4 mg/100 ml. These data support the relative ruminal ammonia values of animals consuming either biuret or urea (Johnson and McClure, 1964 and Oltjen et al., 1968). Levels of blood ammonia from unadapted animals consuming either urea or biuret are very similar. It is possible that urea levels in the ration were below the threshold where ammonia levels in the peripheral blood are affected by diet or time of eating.

It may be concluded, that if peripheral blood ammonia-N concentrations are the criteria, toxicity should not be a problem when NPN compounds are fed with poor quality roughages for maintenance rations. Provided the animals are not starved, intake is probably low enough to allow assimilation of the ammonia which is liberated.

III. FACTORS AFFECTING N-UTILIZATION OF CORN SILAGE AND UREA RATIONS

Previous results have indicated that several factors affect the metabolism of N by animals fed NPN. Smith et al. (1960) and Barth (1962) used multiple regression analyses in an attempt to determine the magnitude of factors affecting N-utilization of NPN rations. Both of the above reports describe data obtained from sheep consuming semi-purified diets, and the primary objective was to quantify the adaptation response on N-utilization. The present study involved part of the data from six different digestion and N-metabolism trials in which steers were fed urea-corn silage rations and which were similar in experimental procedure and variable in proportion of ration components and intake. Several parameters were studied to determine their effects on nutrient digestion and N-metabolism when steers were fed these natural diets. Since only one trial was conducted in which the animals were allowed more than a 10-day preliminary period, adaptation was not one of the parameters in the present study.

Simple correlation coefficients between the dependent variables (apparent nutrient digestibility and N-metabolism) and those available as independent variables may be seen in Table X. Discussion of simple correlation coefficients seems unwarranted because of interdependence of 'independent variables'. However, when multiple regression techniques are used, the effects of the other 'independent variables' are held constant, and the relationships between dependent and independent variables become more meaningful.

TABLE X

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN DIGESTION AND UTILIZATION DATA AND RATION CONSTITUENTS, INTAKE PARAMETERS AND GENERAL ANIMAL PERFORMANCE^a

Item	Apparent digestion coefficients %		True N digest. % ^b	% Absorbed N retained ^c	% Intake N retained ^d	Net protein utiliz. ^e %	Net protein value %
	Dry matter	N					
Dry matter intake	0.004	-.401	0.018	0.173	0.030	0.045	-.087
N intake	-.436	0.055	-.647	-.014	0.181	0.027	0.410
NPN intake	0.106	-.059	0.137	-.238	-.286	-.225	-.392
Digestible energy intake	0.323	-.038	0.227	-.009	-.019	-.025	-.176
Crude fiber intake	0.207	-.122	0.428	-.208	-.360	-.269	-.545
Ration dry matter, %	0.496	-.417	0.556	0.559	0.286	0.390	-.016
Ration nitrogen, %	-.198	0.302	-.337	0.125	0.365	0.239	0.566
Ration NPN, %	0.108	0.044	0.141	-.274	-.289	-.232	-.362
Ration energy, mcal/kg	0.058	-.041	0.133	0.242	0.201	0.188	0.180
Ration fiber, %	0.168	-.085	0.455	-.191	-.358	-.237	-.518
Ration digestible NFE, %	0.480	-.085	0.489	0.112	0.026	0.077	-.202
Readily available energy, mcal/g	0.436	-.486	0.458	0.559	0.284	0.368	0.002
Calorie to protein ratio, kcal/g	0.510	-.469	0.783	0.166	-.210	-.021	-.574
Ration N supplied by NPN, %	0.242	-.097	0.343	-.133	-.253	-.143	-.431
Animal weight gain, kg	0.114	-.052	-.353	0.306	0.219	0.288	0.111

^a $r > .273$, $P < .05$ ($d/f = 55$).

^r $r > .354$, $P < .01$.

^bTrue N digestibility = $\frac{\text{intake N} - (\text{fecal N} - \text{MFN})}{\text{intake N}}$.

^cThomas Mitchell biological value of protein = $\frac{\text{intake N} - (\text{fecal N} - \text{MFN}) - (\text{urinary N} - \text{EUN})}{\text{intake N} (\text{fecal N} - \text{MFN})}$.

^dIntake N retained = $\frac{\text{intake N} - \text{fecal N} - \text{urinary N}}{\text{intake N}}$.

^eNet protein utilization = percent absorbed N retained X true N digestibility/100.

^fNet protein value = NPU X percent crude protein of ration/100.

^gEnergy from grain and plant meal supplements of rations.

For the multiple regression equations, six to nine possible components (independent variables, Table X) were selected by subjective evaluations of magnitude of correlation coefficients and biological importance. They were used in multiple regression equations and ranked in combinations in decreasing magnitude of the coefficient of determination (R^2). The best equations containing four components were used.

Nutrient Digestibility

A summary of the data used in the equations for apparent digestion coefficients is presented in Table XI. The regression equations along with other statistical information are presented in Table XII. The dependent variables, apparent digestibility of dry matter (Y_a), nitrogen (Y_b) and crude fiber (Y_c) were regressed on the following independent variables:

X_1 , calorie to protein ratio in kcal/gm;

X_2 , grams of nitrogen consumed per day;

X_3 , percentage digestible NFE of ration;

X_4 , percentage dry matter of ration.

The regression of dry matter digestibility (DDM) on these four factors accounted for 51 percent of the total variation, and was highly significant ($P < .001$). Highly significant ($P < .01$) effects were exhibited by all except calorie to protein ratio, which contributed significantly ($P < .05$).

The simple relationship between dry matter digestibility and calorie to protein ratio indicated that increasing the calorie to protein

TABLE XI

SUMMARY OF MEAN OBSERVATIONS USED FOR THE CALCULATION OF MULTIPLE LINEAR
REGRESSION EQUATIONS FOR APPARENT DIGESTION COEFFICIENTS

Trial	Number of Animals	Digestion coefficients				Calorie to protein ratio X ₁ kcal/gm	Nitrogen intake gm/day X ₂	Digestible NFE X ₃ %	Ration dry matter X ₄ %
		Dry matter	Nitrogen	Crude fiber					
		Y _a %	Y _b %	Y _c %					
H72T8-1	2	74.1	71.0	65.5	30.5	105.8	48.0	56.6	
H72T8-2	3	72.9	65.0	66.5	33.3	78.9	50.4	52.0	
H72K2-2	4	67.5	50.2	54.2	32.1	99.3	47.9	49.3	
H72K2-3	4	70.2	59.5	56.4	30.1	104.1	48.0	48.9	
H72K2-4	4	72.8	69.1	60.1	28.0	102.2	51.1	54.8	
H72K2-6	4	72.5	68.6	64.9	27.5	107.8	49.5	55.3	
H71K3-1	4	75.2	64.0	75.3	34.6	79.0	50.5	53.5	
H71K3-2	4	74.4	58.3	75.4	37.4	72.8	47.6	57.5	
H71K3-3	4	74.6	60.4	77.3	35.4	72.6	43.4	57.2	
H71K3-4	4	77.9	68.8	80.3	36.3	80.3	55.7	50.8	
H71K3-5	3	73.9	62.2	74.0	38.2	82.7	61.4	53.9	
H71K3-6	4	74.8	64.1	75.4	36.8	78.6	53.9	51.9	
H71K3-7	4	67.3	68.9	55.3	27.3	87.3	39.7	33.1	
H71K3-8	4	70.3	72.4	59.0	27.3	87.4	42.0	33.2	
H71K3-9	3	70.4	73.5	60.0	26.7	89.3	42.6	33.3	
Means		72.5	64.8	66.7	32.1	88.4	48.6	49.3	

TABLE XII
RESULTS OF STATISTICAL STUDY OF COEFFICIENTS OF APPARENT DIGESTIBILITY^{abcd}

Item	Digestion Coefficients		
	Dry matter	Nitrogen	Crude fiber
Coefficient of determination, $R^2 = \epsilon \hat{y}^2 / \epsilon y^2$	.513	.463	.739
Mean square due to regression: $\epsilon \hat{y}^2 / 4$:	99.15***	273.78***	879.18***
Mean square due to deviations from regression, $\epsilon d^2 / 50$:	7.54	25.42	24.83
Standard partial regression coefficients:			
$b'y1.234$	-0.413	-1.080	0.134
$b'y2.134$	-0.645	-0.599	-0.535
$b'y3.124$	0.427	0.527	0.223
$b'y4.123$	0.478	-0.082	0.321
Standard error of regression coefficients (d/f = 50):			
$S_{by1.234}$	0.192; t = 1.99*	0.353; t = 4.94***	0.348; t = 0.88
$S_{by2.134}$	0.047; t = 4.13***	0.086; t = 3.65***	0.085; t = 4.67***
$S_{by3.124}$	0.084; t = 3.20**	0.154; t = 3.76***	0.152; t = 2.29*
$S_{by4.123}$	0.064; t = 3.32**	0.117; t = 0.54	0.116; t = 3.05**
Regression equations:			
$\hat{Y}_a = 78.35 - 0.381X_1 - 0.193X_2 - 0.268X_3 + 0.212X_4$			
$\hat{Y}_b = 123.32 - 1.74X_1 - 0.313X_2 + 0.579X_3 + 0.064X_4$			
$\hat{Y}_c = 57.49 + 0.307X_1 - 0.396X_2 + 0.348X_3 + 0.352X_4$			

^a X_1 = Calorie to protein ratio, kcal/g.

^b X_2 = Nitrogen intake, g/day.

^c X_3 = Digestible NFE, %.

^d X_4 = Ration dry matter, %.

* (P < .05).

** (.05 < P < .01).

*** (.01 < P < .001).

ratio would increase DDM. However, when N intake, digestible NFE and percent dry matter were held constant, the relationship was negative. Nitrogen intake was also negatively associated with DDM. The reason for this negative b value is that higher N intake is usually the result of higher dry matter intake, and increased dry matter intake has often been associated with a decreased DDM due to rapid passage of materials through the digestive tract (Forbes et al., 1928 and Andersen et al., 1959). For each percentage unit increase in digestible NFE in the ration, there was a resulting increase of 0.268 percentage units in dry matter digestibility. Asplund and Harris (1972) observed that dry matter digestibility increased from 59 to 86 percent when the diet was alfalfa hay or beet pulp, respectively, and which had NFE contents of 66 and 94 percent respectively. The greater solubility of starch over cellulose and the greater ease by which it is hydrolyzed influences the digestibility of combined nutrients (dry matter), especially when it composes a large fraction.

The regression of apparent N digestibility on the four dependent variables accounted for 46 percent ($P < .001$) of the total variability in N digestibility. The contribution of calorie to protein ratio (X_1), N intake (X_2) and percent digestible NFE (X_3) were each highly significant ($P < .001$). Each unit increase in calorie to protein ratio resulted in 1.74 percentage units decrease in N digestibility, when the other independent variables were held constant. Negative relation between N digestibility and the ratio of calories to protein results in a positive relation between protein content and apparent N digestibility. Holter and Reid

(1959) noted that apparent N digestibility increased with increasing concentrations of crude protein in the ration. The daily amount of intake N was also negatively associated with N digestibility. With the assumptions that N digestibility is directly related to dry matter digestibility and the N intake is associated with dry matter intake, this relationship can be understood. As noted before, increased dry matter intake is usually related to decreased digestibility of nutrients (Forbes et al., 1928 and Andersen et al., 1959).

The regression of crude fiber digestibility on the four independent variables accounted for 74 percent of the total variation ($P < .001$). Crude fiber digestibility was positively related to calorie to protein ratio. This might be explained by the negative relationship between the crude fiber and crude protein content of feeds, especially forages. If crude protein decreases (e.g., increase in calorie to protein ratio), crude fiber increases (Coleman et al., 1971 and Lane, 1972), and increased crude fiber content is usually associated with increased crude fiber digestibility (Smith et al., 1960 and Harris, Kearl and Fannesbeck, 1972). Nitrogen intake was associated negatively with crude fiber digestibility, both singly ($r = -.647$) and collectively when N intake was examined with three other variables. This might be explained by the same reasoning as that used for N digestibility. The absolute standard partial regression coefficient was highest for N intake indicating that it was the most important contributor to the equation ($P < .001$).

N-Metabolism

Three dependent variables were chosen to represent intermediary N metabolism. They were: 1) percent absorbed N retained as calculated by the Thomas-Mitchell method (Y_d); 2) percent intake N retained which includes digestibility (Y_e); and 3) net protein utilization (Y_f), which includes digestibility but is adjusted for metabolic fecal and endogenous urinary nitrogen. A summary of the data used is presented in Table XIII. These dependent variables were regressed on the following independent variables:

- X_1 - percent N supplied by urea;
- X_2 - energy supplied by concentrates, mcal/day;
- X_3 - N intake, g/day; and
- X_4 - fiber intake, kg/day.

The regression equations and other statistical results for relating N utilization to these four independent variables are in Table XIV. Of the total variation in percentage of absorbed N retained, intake N retained and net protein utilization, 34, 18 and 20 percent was accounted for by their regression on the independent variables.

With other independent variables held constant, percentage of nitrogen supplied by urea was essentially not related to any of the three measures of N utilization. Barth (1962) also found this to be the case when sheep were fed semi-purified diets and when urea supplied approximately 75 percent of the total N. However, Smith et al., (1960) noted that increasing the N supplied by urea decreased retention of

TABLE XIII

SUMMARY OF MEAN OBSERVATIONS USED FOR THE CALCULATIONS OF MULTIPLE LINEAR REGRESSION EQUATIONS FOR NITROGEN METABOLISM.

Trial	No. of steers	Retention of absorbed Na Y _d %	Retention of intake N ^b Y _e %	Net Protein utilization ^c Y _f %	N supplied by NPN X ₁ %	Energy from concentrates X ₂ mcals/day	Nitrogen intake X ₃ g/day	Fiber intake X ₄ kg/day
H72T8-1	2	50.1	21.6	42.3	28.9	9.36	105.8	0.812
H72T8-2	3	54.8	19.1	44.1	38.7	9.36	78.9	0.585
H72K2-2	4	54.2	12.6	36.5	32.3	10.4	99.3	0.767
H72K2-3	4	63.2	25.8	47.0	30.6	10.4	104.0	0.756
H72K2-4	4	79.3	45.3	64.8	22.3	11.3	102.2	0.518
H72K2-6	4	72.6	39.7	59.0	21.1	11.3	107.8	0.515
H71K3-1	4	60.8	24.4	48.1	28.8	10.6	79.0	0.806
H71K3-2	4	74.7	27.6	53.5	47.4	10.5	72.8	0.932
H71K3-3	4	71.0	29.4	55.2	62.7	10.5	72.6	0.926
H71K3-4	4	64.6	27.5	49.9	27.6	10.6	83.0	0.892
H71K3-5	4	49.5	12.6	38.5	41.5	10.5	82.7	0.913
H71K3-6	4	68.3	29.9	54.9	61.8	10.4	78.6	0.977
H71K3-7	4	55.4	23.8	46.3	24.3	3.73	87.3	0.708
H71K3-8	4	51.9	24.1	44.6	34.8	3.74	87.4	1.021
H71K3-9	3	44.1	17.3	38.2	45.4	3.74	89.3	0.793
Means		62.0	36.0	48.8	36.5	9.16	88.4	0.795

^aThomas-Mitchell biological value of protein = $\frac{\text{intake N} - (\text{fecal N} - \text{MFN})}{\text{intake N} - (\text{fecal N} - \text{MFN})} - (\text{urinary N} - \text{EUN})$.

^bPercent intake N retained = $\frac{\text{intake N} - \text{fecal N} - \text{urinary N}}{\text{intake N}}$.

^cNet protein utilization = percent absorbed N retained X true digestibility/100.

TABLE XIV

SUMMARY OF STATISTICAL EVALUATIONS FOR NITROGEN METABOLISM DATA^{abcd}

Item	Absorbed N retained Y_d	Intake N retained Y_e	Net protein utilization Y_f
Coefficient of determination, $R^2 = \frac{\sum \hat{Y}^2}{\sum Y^2}$	.342	.178	.198
Mean square due to regression $\frac{\sum \hat{Y}^2}{4}$:	598.8***	239.8*	225.1*
Mean square due to deviations from regression, $\frac{\sum d^2}{50}$:	92.1	88.5	73.0
Standard partial regression coefficients: $b'y_1.234$	0.070	0.036	0.080
$b'y_2.134$	0.519	0.215	0.306
$b'y_3.124$	-.131	-.009	-.135
$b'y_4.123$	-.227	-.347	-.338
Standard error of regression coefficients ($d/f = 50$): $S_{by1.234}$	0.148; $t = 0.40$	0.146; $t = 0.19$	0.132; $t = 0.42$
$S_{by2.134}$	0.494; $t = 4.31***$	0.484; $t = 1.60$	0.439; $t = 2.30^*$
$S_{by3.124}$	0.146; $t = 0.81$	0.143; $t = 0.05$	0.130; $t = 0.75$
$S_{by4.123}$	11.456; $t = 1.42$	11.234; $t = 1.95^*$	10.202; $t = 1.92^*$
Regression equations: $Y_d = 63.68 + 0.060X_1 + 2.128X_2 - 0.118X_3 - 16.33X_4$			
$Y_e = 35.98 + 0.027X_1 + 0.773X_2 - 0.007X_3 - 21.86X_4$			
$Y_f = 61.70 + 0.055X_1 + 1.011X_2 - 0.098X_3 - 19.61X_4$			

^a X_1 = Percent N supplied by urea.^b X_2 = Energy supplied by concentrates, mcal/day.^c X_3 = Nitrogen intake, g/day.^d X_4 = Fiber intake, kg/day.* = ($P < .05$).** = ($.05 < P < .01$)*** = ($.01 < P < .001$)

absorbed N. They also fed semi-purified diets to sheep and urea supplied approximately 60 percent of the total dietary N. Also, Chalupa, Evans and Stillions (1964) noted that utilization of N by steers decreased as the percentage N supplied by urea in semi-purified diets decreased.

Two primary differences existed between the nature of the rations of the present study and those discussed by Smith et al., (1960), Barth (1962) and Chalupa, Evans and Stillions (1964). In the present study, the rations contained natural components. Further, a smaller portion of the total dietary N was supplied by NPN. Chalupa (1968) in a review, noted that if NPN compounds furnished essentially all of the supplementary nitrogen in natural diets, usually only one-third of the total N would be supplied by NPN. There were occasions in the present study, though, when urea furnished as much as 60 percent of the total N.

The amount of energy supplied by concentrates exhibited a significant ($P < .001$) effect on retention of absorbed N. An increase of one megacalorie in energy from concentrates resulted in 2.13 percentage units increase in retention of absorbed N. Barth (1962) also noted that increasing readily available carbohydrates increased N utilization. Further, Fontenot et al. (1955) observed that retention of N by steers consuming wintering rations containing 10 percent crude protein was greater after cerelose was added to the rations. However, urea was not a ration component. They explained this phenomenon as a sparing action of the energy on protein. In a review, McLaren (1964) suggested the response may be due to increased microbial protein synthesis as a

result of the availability of excess carbohydrates or intermediates of carbohydrate metabolism at the time of ammonia release from urea hydrolysis. Net protein utilization was also significantly ($P < .05$) increased by an increase in energy supplied by concentrates. Nitrogen intake did not significantly affect any of the measures of N metabolism. This may indicate that N intake of the animals was within the optimum range required for maintenance and growth without activity.

The regression of retention of absorbed N on fiber intake with the other variables held constant, was not significant ($P < .05$). However, there was a large negative regression coefficient indicating that increasing fiber intake decreased retention of absorbed N. When percentage of intake N retained and net protein utilization were regressed on fiber intake, the contribution toward the coefficient of determination was significant ($P < .05$). This may be explained by the adverse effect of fiber on nitrogen digestibility, which is included in both of these measures.

V. SUMMARY

Two successive 7-day total-collection digestion and nitrogen (N) metabolism trials were conducted with beef steers to compare biuret with urea and to determine if other ration constituents influence utilization of N furnished by biuret. Twelve medium grade Hereford steers averaging 221 kg in body weight were allotted to one of the following treatments: 1) urea control; 2) biuret control; 3) biuret-molasses; and 4) biuret-corn. All animals received a basal ration of

poor-quality fescue hay and were allowed 42 days to adapt to the NPN source before the start of the first trial.

A comparison of the data from the two trials indicated nutrient digestibility (dry matter, crude protein and gross energy) was not affected by NPN source. However, the addition of readily-fermentable energy (molasses, cornmeal) increased digestibility of all nutrients. Dry matter and gross energy digestibility of rations containing biuret and additional energy were significantly ($P < .10$) higher than that of the urea and biuret control rations. Nitrogen digestibility was non-significantly higher in rations containing additional energy than in both control rations.

The percent absorbed N retained by animals consuming all types of biuret rations was lower than that by animals consuming the urea ration. The difference was from 6 to 8 percentage units, but was not significant ($P < .10$). Net protein utilization and percent intake N retained, which take into account digestibility, followed essentially the same patterns as percent absorbed N retained. They did, however, reflect the advantage of additional energy on digestibility.

Another experiment was conducted to determine the pattern of peripheral blood ammonia and urea as a function of time after feeding. Six animals, used previously in metabolism trials, and six unadapted to NPN, were allotted to one of the following treatments: 1) urea-adapted; 2) biuret-adapted; 3) urea-unadapted; or 4) biuret-unadapted. Rations consisted of long timothy hay, blackstrap molasses and either urea or biuret. NPN comprised approximately 1.5 percent of the ration.

Jugular blood samples were taken at intervals of 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 6 hours after feeding. An incomplete Latin Square design was used for sampling. Adapted animals consuming urea had the highest blood ammonia levels. Blood from animals of other treatments all contained approximately the same level of ammonia within all treatments, peak ammonia-N levels appeared at 1 hour after feeding. Plasma urea levels were fairly constant for all animals, averaging approximately 12-14 mg per 100 ml plasma. There was, however, a gradual increase in plasma urea from 2 to 6 hours after feeding.

✓ Data from six total-collection digestion and N-metabolism trials with steers were compiled to examine some factors affecting ration digestibility and N-utilization of urea-corn silage based rations. F-max tests indicated homogeneity of variances among the trials. Multiple regression equations were developed to determine the effect of various ration constituents and intake parameters on nutrient digestibility and efficiency of N-metabolism. Calorie to protein ratio, grams N intake, percent digestible NFE and ration dry matter content together accounted for 51, 46 and 74 percent of the total variability in the apparent digestibility of dry matter, crude protein and crude fiber, respectively. Increasing calorie to protein ratio resulted in a decrease of apparent digestibilities of dry matter and nitrogen, and an increase in digestibility of crude fiber. Nitrogen intake was associated negatively with digestibility. Increasing ration dry matter content increased dry matter and crude fiber digestibility and decreased that of crude protein.

When percent absorbed N retained, percent intake N retained and net protein utilization were the dependent variables, their regression on the four independent variables: 1) percentage of ration N supplied by urea; 2) energy supplied by concentrates; 3) N intake; and 4) fiber intake accounted for 34, 18 and 20 percent respectively, of the total variability in the three dependent variables. Percentage of N supplied by urea and N intake had no significant effect on any measure of N-metabolism. When energy supplied by concentrates increased from 3.8 to 11.3 mcal/day, retention of absorbed N, retention of intake N and net protein utilization increased 15.7 (significant, $P < .001$), 5.7 and 7.5 (significant, $P < .05$) percentage units, respectively. Increased fiber intake was associated with significantly ($P < .05$) decreased retention of intake N and net protein utilization. There was little effect of fiber intake on retention of absorbed N.

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APPENDIX

TABLE XV
STATISTICAL ANALYSIS FOR COEFFICIENTS OF DIGESTIBILITY

Source of variation	Degrees of freedom	Mean Squares			
		Apparent digestibility, %			True N digestibility, %
		DM	CP	GE	
Total	17				
Tmt	3	47.0	26.8	58.5	9.35
(1 vs 2)	1	0.3	0.3	3.0	10.97
(3 vs 4)	1	0.2	0.6	0.04	2.25
(1+2 vs 3+4)	1	138.9*	41.4	167.9*	12.14
Error 1	5	29.7	43.3	36.2	7.96
Trial	1				
Treatment X Trial	3	1.0	0.4	17.7	1.55
Error 2	5	41.6	18.9	39.0	1.24
		33.8	30.1	34.8	0.67

*P < .10

TABLE XVI

STATISTICAL ANALYSIS FOR N UTILIZATION

Source of variation	Degrees of freedom	Mean Squares			
		Absorbed N retained	Net protein utilization	Intake N retained	Net protein value
Total	17				
Treatment	3	70.7	58.8	28.2	0.76
(1 vs 2)	1	125.2	139.1	61.1	1.87
(3 vs 4)	1	13.4	6.8	15.3	0.05
(1+2 vs 3+4)	1	71.3	31.1	7.0	0.38
Error 1	5	76.0	79.2	109.5	0.74
Trial	1				
Treatment X Trial	3	92.0	86.0	103.5	0.48
Error 2	5	54.1	40.3	113.2*	0.37
		23.8	18.8	27.3	0.23

*P < .10

TABLE XVII
NUTRIENT DIGESTIBILITY AND N-UTILIZATION
OF INDIVIDUAL ANIMALS

Ration and animal number	Digestibility coefficients %			Absorbed N retained, %	Net protein utilization	Intake N retained, %
	DM	CP	GE			
Trial I						
Urea control						
2	54.4	59.6	51.9	67.1	61.4	20.9
3	38.7	45.1	35.7	61.4	55.0	1.79
Biuret control						
4	42.1	49.4	39.1	55.7	48.2	0.61
5	44.0	54.4	41.1	55.4	49.0	4.25
Biuret-molasses						
8	55.7	57.9	52.6	57.6	50.9	10.8
9	53.0	57.1	50.8	71.1	64.4	22.0
Biuret-corn						
10	56.6	62.4	54.0	63.4	58.5	16.8
11	53.3	54.6	50.9	55.9	48.7	5.48
12	54.1	60.6	52.0	49.4	44.5	3.49
Trial II						
Urea control						
1	49.1	52.1	48.8	65.0	57.3	11.1
2	49.4	56.1	48.0	62.0	55.2	12.8
Biuret control						
4	45.4	51.1	43.3	51.7	43.6	-0.85
5	53.1	59.1	50.7	59.2	52.4	12.9
Biuret-molasses						
8	48.7	50.3	47.2	42.5	37.3	-10.9
9	51.6	56.4	51.6	55.4	50.2	6.27
Biuret-corn						
10	47.5	54.7	47.4	60.0	55.3	4.66
11	47.7	51.5	46.1	49.8	44.2	-4.47
12	52.5	60.0	52.1	47.3	43.0	1.13

TABLE VIII

DRY MATTER INTAKE, NITROGEN BALANCE AND URINARY
N PARTITION FOR INDIVIDUAL STEERS

Ration and animal number	Dry matter intake	Nitrogen, g/day			Urinary N, g/day		
		intake	fecal	urinary	urea	creatinine	creatinine
					+ NH ₃		
Trial I							
Urea control							
2	4.07	70.7	28.1	27.8	24.6	0.084	0.968
3	3.91	65.8	35.4	29.2	21.6	0.180	0.826
Biuret control							
4	3.88	69.7	36.2	33.1	20.8	0.155	0.716
5	3.57	67.6	31.7	33.0	23.3	1.677	0.269
Biuret-molasses							
8	3.93	67.7	28.6	31.8	20.2	0.095	0.932
9	4.53	75.7	32.5	26.7	19.3	2.008	0.000
Biuret-corn							
10	3.68	60.7	23.7	26.8	20.9	0.179	0.912
11	4.58	75.5	35.3	36.0	26.0	2.145	0.143
12	3.58	63.4	25.9	35.3	28.4	0.095	1.340
Trial II							
Urea control							
1	3.28	56.7	26.6	23.9	16.6	0.090	0.517
2	3.40	63.9	27.5	28.3	19.9	0.083	0.817
Biuret control							
4	3.52	65.8	33.1	33.3	22.4	0.174	0.587
5	3.83	70.4	29.5	31.8	21.3	0.172	0.852
Biuret-molasses							
8	3.71	60.3	30.1	36.8	22.7	0.124	1.037
9	4.31	72.3	31.6	36.2	24.0	0.135	0.831
Biuret-corn							
10	3.46	55.1	26.0	26.6	17.1	0.075	1.155
11	4.43	71.2	35.6	38.8	25.7	0.725	0.692
12	3.82	66.9	28.0	27.0	26.0	0.544	1.186

VITA

Samuel Winfred Coleman was born in Carroll County near Huntingdon, Tennessee on March 7, 1947. He attended elementary school in McLemoresville, Tennessee and high school at Trezevant High School, where he graduated in 1965. In September of that year, he entered the University of Tennessee at Martin and received a Bachelor of Science degree in Agriculture in 1969. While an undergraduate student, he was a member of the University Marching Band and was a member of the Pi Sigma Phi Honorary Society. He was also selected to Who's Who in American Colleges and Universities.

In September, 1969, he received a National Defense Education Act Fellowship and began graduate study in the Department of Animal Husbandry at the University of Tennessee. He received the degree of Master of Science with a major in Animal Husbandry in March, 1971.

He is a member of the American Society of Animal Science, Gamma Sigma Delta, Phi Sigma and Alpha Zeta. He was commissioned second lieutenant in the U. S. Army Reserve in June, 1971, upon completion of the ROTC program.

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